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BULLETIN NO. 286.

(Research Bulletin)

The Pathology and Bacteriology of the Reproductive Organs of Mares in Relation to Sterility.

By W. W. DIMOCK and P. R. EDWARDS

Of all the difficulties that may be encountered in livestock production, loss from disease probably stands first in economic importance. In highly developed, well-bred herds, functional disturbances and diseases of the reproductive organs resulting in sterility constitute a problem that demands serious consideration.

Much of the early work on the general handling of breeding problems was misdirected effort. Many sound practices specifically indicated for certain conditions have been applied to cases of sterility for which they were not indicated. The first and vital point to be observed in handling sterility is a correct diagnosis; and second, the use of agents and practice of methods the value and effect of which have been previously determined as basically applicable for a particular condition. Equally important is the early recognition of incurable cases, in order that they may be promptly removed from the herd.

Those who have been actively engaged in horse breeding as an industry realize fully that sterility of mares, impotency of stallions and the loss of foals from prenatal infections are among their most serious problems. The successful control and prevention of these conditions must necessarily be based upon a thoro knowledge of the causes, characteristics of the disease and all contributing factors. This information can only be gained from a systematic study of a large number of cases.

The data herein reported result from a study of breeding problems as set forth in the title and as they actually occur on farms in central Kentucky. The work has covered a period from July, 1921, to June, 1927. In the conduct of this work materials were obtained from, and observations made on, animals living under normal conditions of breeding farms in central Kentucky. Because of this it became necessary to arrange with the owners a method of procedure that would provide for frequent and year-round observations and at the same time not interfere with the general policies of management of the farm. The plan which was adopted has previously been published and is now quite generally followed. The object of this method of procedure is that accurate knowledge of the condition of the genital tract of each brood mare maintained in a breeding establishment may be obtained. The most logical time to start making observations is in the fall two to three months following the close of the breeding season. At this time all brood mares that have been bred must be examined for pregnancy. Those mares found pregnant should be vaccinated against infectious or contagious abortion and then handled, fed and cared for according to the best known methods. The genital tract of each barren mare should be thoroly examined clinically and bacteriologically and the mares separated into infected and non-infected groups. Many of the non-infected mares will be found to be normal and therefore may be turned into winter quarters. The infected mares will require proper treatment for a period of weeks or months.

Methods of Clinical and Bacteriological Examination.

For the purpose of examination the mare should be confined only sufficiently to protect the examiner; the tail bandaged; the external genitals washed with soap and water, and rinsed with antiseptic solution. In the routine examination of mares for pregnancy and clinical examination for diagnosis, including the taking of cultures, two buckets of antiseptic solutions are considered sufficient. When a number of infected mares on the

same farm are under treatment and being examined two or three times a week, it is advisable to have three buckets, one for washing, one for rinsing, and one for instruments. The antiseptic we have used almost altogether has been one of the chlorinated preparations. These are preferred because they are non-irritating and antiseptically efficient. The solution in the buckets should be changed as conditions indicate.

An examination of the whole genital tract is to be made. This is to include a detailed rectal examination of the uterus, ovaries and tubes, if palpable. A vaginal speculum is inserted to make possible a visual examination of the vagina and external cervix. Material for bacteriological examination is obtained from the cervix and uterus by using a five-inch platinum needle attached to a twenty-inch holder. The needle is flamed, passed thru the vagina and os uteri into the uterus, moved back and forth two or three times and withdrawn. If the needle has touched nothing after removal from the os uteri it is used to inoculate a tube of culture medium. The practice has been to inoculate two tubes of culture medium, using fresh material for each tube. With a little practice, entirely reliable cultures can be obtained in this way. Unfavorable surroundings should be avoided and every sanitary precaution observed.

During the past five years approximately four thousand mares have been examined for pregnancy. Of this number 1,606 were barren. A complete clinical examination was made of the reproductive organs of these barren mares, supplemented by one or more bacteriological examinations, averaging 2.42 for each mare, making a total of 3,898. In the large majority of cases the bacteriological findings confirmed the clinical evidence of infection. As a result of the 3,898 bacteriological examinations 36.55 per cent of all barren mares examined were found to be suffering from inflammatory changes, in the genital tract, due to infection.

Of the 1,606 barren mares examined, all except a few maiden mares, had previously been bred and had failed to conceive. Upon this number (1,606) the percentages of non-

infected and infected mares are based: The percentages of mares suffering with different types of infection also, are based upon this number.

Total number of barren mares examined	1,606	100%
Total number of barren mares not infected	1,019	63.45%
Total number of barren mares infected	587	36.55%
Total number of barren mares with Streptococcus metritis	390	24.28%
Total number of barren mares with Encapsulatus metritis	60	3.74%
Total number of barren mares with miscellaneous infections	137	8.53%

Of the total number of barren mares examined, 1,019—or 63 per cent—yielded a negative bacteriological examination each time they were cultured, and are considered not infected.

Of the 587 mares which are considered infected, many were repeatedly examined and yielded a positive bacteriological result on one or more times that cultures were taken. The findings may be summarized as follows:

Total number with *Streptococcus genitalium*	390	66.44%
Total number with *Encapsulatus genitalium*	60	10.22%
Total number with miscellaneous infections	137	23.34%
Total number infected	587	100.00%

In handling and studying barren mares in the field they are first divided into two main clinical groups: (1) infected mares, and (2) non-infected mares. The infected mares are further classified on the basis of the type of infection found on bacteriological examination, as follows:

Group one (a) Mares infected with *Streptococcus genitalium.*
Group one (b) Mares infected with *Encansulatus genitalium.*
Group one (c) Mares infected with miscellaneous microorganisms.

The non-infected barren mares are further classified into seven divisions on the basis of clinical examination only. This classification is essential if the mares are to receive attention before the beginning of the next breeding season. In most of our work we have found it satisfactory and convenient to classify the non-infected barren mares as follows:

Group two (a) Mares apparently normal.
Group two (b) Mares with abnormally large cystic ovaries.
Group two (c) Mares with abnormally small ovaries.
Group two (d) Mares physiologically exhausted.
Group two (e) Mares with ovarian and uterine tumors.
Group two (f) Mares with chronic cystic degeneration of the uterine wall.
Group two (g) Mares with cystic degeneration and chronic fibrosis of the ovaries.

While this classification is based largely on clinical findings, the breeding record and regularity of periods of estruation are always of great help in making the final decision. Further, post-mortem examinations on cases that had been previously classified clinically revealed evidence that is on the whole confirmatory of the general correctness of this classification.

It has not been possible to learn the subsequent breeding record of all the 1,019 non-infected barren mares examined. Failure to conceive was in most cases apparently seasonal and temporary, as it is known that a considerable number conceived the following year and gave birth to normal foals. Many are known to have remained barren for a number of years but later conceived. Some have died, and others have been destroyed. Of those destroyed, the cause of sterility was determined as being due to structural and functional changes in either the ovaries or uterus; no infection was found. A limited number had ovarian and uterine tumors. A few of the early cases observed are still barren, and altho bred every year for the last five or six years, have not conceived, yet were free from infection at last examination.

It has not been possible to keep all the infected mares under observation, but in those cases where it has been possible to learn the outcome, a fairly definite estimate can be made. Many of these mares have been destroyed or died from various causes. Many are still barren and show infection, including mares that were treated and those that were not. Some have recovered and become regular producers. In this group are mostly mares that were treated; however, a very few that were not treated have since produced healthy foals. Some that were

bred and conceived, aborted or gave birth to diseased foals. A few that were treated have ceased to show infection, but are non-breeders.

Clinical Pathology of the Genital Tract in Cases of Infection With *Streptococcus genitalium.*

The clinical picture of uterine infection caused by *Streptococcus genitalium* varies somewhat in individual cases, and also according to the duration of infection. Infected mares are often soiled with pathological exudate over the vulva and perineal region. The tail and hair of the quarters adjacent to the external genitals are soiled and matted. The vulva may gape with a slight protrusion of the mucous membrane. Some cases are badly sunken in the perineal region, showing marked loss of tone in the muscle and other supporting structures of the vulva and vulvo-vaginal canal. Upon inspection of the genital tract the mucous membrane is found congested and moist, blood vessels are distended, tortuous and stand out in great prominence. A purulent exudate is found along the canal and in the vaginal cavity. The vaginal mucous membrane shows evidence of a superficial inflammation. A considerable quantity of exudate is often present in the vagina; it may be a very thin, slightly cloudy muco-purulent material or of a thick yellowish white character containing many flocculi. Again there may be no collection of exudate in the vagina, but in such cases a very thin, clear, slightly frothy exudate will be observed coming from the cervical canal. A small quantity of this material can be scooped up on the spoon of the speculum. In the very great majority of normal barren mares the vaginal mucous membrane, including the vaginal cervix, is dry, pale, and coated with a grayish white, dry, gummy secretion. Recently infected cases present a typical picture of acute endocervicitis and endometritis. The cervix is congested and moist, the mucous membrane swollen and glistening with the folds greatly enlarged, and the os uteri not as firmly closed as in normal mares. The secretion is usually thin, slightly viscid, and opaque, containing a few

leucocytes and epithelial cells. Infected mares show a hypersecretion during the estrual period, which may subside appreciably during the anestrual period.

It is not always possible to distinguish between an acute and a chronic infection on clinical examination. In some cases which have continued for a considerable time the clinical picture may still resemble an acute infection while, on the other hand, recently infected cases may resemble the chronic form, these variations depending upon the age of the mare and the condition of the uterus at time of infection, young mares frequently presenting the former appearance, and old mares the latter.

In chronic cases of uterine infection with *Streptococcus genitalium* the exudate is decidedly muco-purulent, always present in a greater or smaller amount, the vulva, the inner surfaces of the thighs and the tail being soiled with dried exudate. Inflammation is evident from the vulva to the ends of the horns of the uterus. The cervix varies in its reaction to the chronic inflammation. In certain cases it is greatly dilated, its conformation is lost, and it exists as a mere membranous border to the uterine cavity; in other cases the cervix is enlarged, elongated, and indurated, its canal lined with a smooth, firm membrane, in striking contrast to the mucous membrane of the normal cervical canal, while in a few cases adhesions occur closing the cervix and resulting in pyometra. In those cases where the uterine wall remains fairly well contracted the exudate is readily expelled, while with a dilated and paretic uterus the exudate accumulates in the uterine cavity. In cases of long standing the folds of the uterine mucosa may disappear, the mucosa is denuded over large areas and is replaced by cicatricial tissue. Inoculation of culture tubes from the cervix and uterus is a routine part of the clinical examination and is essential *in many cases* to differentiate between infected and non-infected mares and to determine the type of infection responsible for the inflammation.

Perineal injuries which occur at time of parturition are of rather frequent occurrence. They vary in position and extent

and always predispose to infection of the genital tract. The most frequent location for lacerations of the vulva is at the upper commissure, extending under and around the anosphincter muscle at an angle of about forty-five degrees from the median line. Tears may occur at a point below the upper commissure and straight to the side, also and quite frequently, directly upward along the median line. Occasionally the laceration extends into the anus and rectum. Tears into the rectum are usually fatal, unless close to the anus. Vulvo-anal ruptures are not necessarily fatal to the mare but are, in most cases, fatal to her as a brood mare. We have records of three mares with vulvo-anal rupture that reproduced; two for one season and one for two seasons; however, they later all became infected, developed a severe streptococcic metritis and were destroyed.

Infected mares, with laceration of the vulva of however limited extent should, before attempting treatment to overcome the uterine infection, first be operated on to correct the tear.

Salpingitis: We have never been able to detect an enlarged oviduct on rectal examination and are of the opinion that salpingitis is very rare in the mare. This opinion is supported by the fact that in the 83 mares upon which post-mortem examinations have been held, the majority being mares with uterine infection, only eighteen showed evidence of salpingitis. This was determined by gross and microscopic examination. Not all the fallopian tubes showing inflammation were positive upon culture.

Gross Pathology. The genital organs available for study on post-mortem were practically all from old mares that had produced several foals. All of them had a history of having been barren at least two years; many of them longer, and in the case of a majority a clinical history of pathological exudation from the genital tract from the first year barren. A number had been treated over a long period of time, others had been bred repeatedly during each breeding season for several years. In the case of a few it was possible to get authentic information on the termination of the last pregnancy. This included abortion, premature birth, difficult parturition, retained placenta,

dead foal, sleeper, foal that died with symptoms of navel ill or joint ill, mare badly bruised or torn at time of foaling and foaled normal. Some of these conditions predispose to infection of the genital tract, others are the result of infection. Concurrent infection is not uncommon. Therefore, in studying the pathology of the genital tract of mares due to infection with streptococcus genitalium, all factors that would tend to modify and exaggerate the final termination of the inflammatory processes have been taken into consideration.

The gross pathological changes are those of congestion, inflammation, infiltration, hemorrhage, exudation, desquamation, degeneration, and proliferation. A combination of these changes usually involves the entire genital tract; however, the main seats of pathological alterations are in the cervix and uterus.

Study of microscopic sections from different parts of the cervix and uterus substantiates the changes found in the gross specimens. Sections from different parts of the same genital were examined and each showed approximately the same type and degree of change, which indicates that the inflammatory processes are rather evenly distributed. Further, each different set of genitals examined also showed the same changes with only slight variations, except in a very few cases in which the terminal pathological picture was very extreme and unusual.

Vaginitis. As an independent condition vaginitis is not common, and almost never severe. It does occur frequently in association with cervicitis and metritis. The exudate from the cervix and uterus is full of microorganisms and inflammatory products which are very irritating to the vaginal mucosa as they pass over it. The inflammatory changes that occur usually clear up very quickly when the cause is removed. Microscopic sections have not been made from the vagina.

Cervicitis. Upon opening the genital tracts of mares it is found that the appearance and condition of the mucosa will vary somewhat in each individual case. In nearly all the cases resulting from this infection, and especially those of long standing, the cervical mucosa is usually greatly congested and has a very deep purplish-red color. The folds are thickened,

pendulous, and often extend into the vagina. The vaginal portion of the cervix is usually greatly dilated. In a few cases the cervical canal has been found tightly closed thru the formation of adhesions. The adhesion may be a false membrane over the external os uteri. As the uterus fills up with exudate the weight pulls the cervix forward and downward, often almost completely obliterating the vaginal cervix, the false membrane closing the canal appears as a continuous part of the vaginal mucosa. In other cases the adhesions are found in the uterine end of the cervix, while in others they may be all thru the canal. Closure of the cervical canal in cases of uterine infection always results in a typical pyometra. The so-called cervical glands, in some cases, are found enlarged and filled with coagulated mucus and cells. If the outlet has become closed thru proliferation of epithelium or connective tissue it results in the formation of a cyst. In case of active infection in the glands, deep suppurative processes and even abscess formation may occur. In our histological study of the cervix, fibrosis has not been found to be as extensive as was indicated at time of post-mortem examination, and particularly not as pronounced as clinical appearance would indicate.

In milder cases the confirmation is good and the mucosa is only slightly discolored. The mucus exudate is scanty, thin, and only slightly clouded. The mucosa is moist, glistening, congested, and has the appearance of early inflammation. In other cases the membrane of the mucosa is of a dirty, pale yellowish color.

In severe cases the color of the exudate may vary considerably; usually, however, the more chronic the condition the greater the quantity of exudate. In some cases a rather thin, milky white, flocculent exudate is seen in the cervix, in others it is of a muco-purulent character. The cervical structure may, as a whole, be enlarged and indurated, showing that extensive fibrosis has taken place, while in other cases it appears normal with the exception of congestion and inflammation of the mucosa.

Microscopic sections of the cervices show inflammatory processes confined mostly to the epithelium. The individual

epithelial cells are degenerated, granular in appearance, with the general outline of each becoming indistinct and the nuclei staining very poorly. When the inflammatory processes have advanced beyond this stage, as they frequently do, the epithelium may be sloughed and replaced by inflammatory products such as degenerated epithelial cells, cell detritus, inflammatory round cells and exudate. The muscularis of the cervix in the cases studied was seldom found involved. A slight amount of fibrosis may be seen which is characterized by increased intermuscular connective tissue and inflammatory round cells scattered thruout these structures.

Metritis. The uterus and horns are the seat of many pathological changes. The most striking feature is, perhaps, the deep purplish red and hemorrhagic areas scattered in irregular-shaped patches over the whole mucous surface. Distinct hemorrhages, in the form of petechiae and ecchymoses, within the discolored areas are often seen. Between these reddened and hemorrhagic areas the mucosa is whitish or pale yellow in color. At many points the mucosa is denuded of epithelium and has a dull granular appearance especially noticeable within the hemorrhagic and reddened areas.

The character and amount of the exudate vary somewhat, but in every case it is a distinguishing part of the inflammation. In those cases which appear as an acute catarrhal endo-metritis the exudate is milky white in color and of a thin muco-purulent character with few flocculi and not excessive in amount. In other cases large quantities of a thick muco-purulent exudate with many flocculi and strings of fibrin overshadow the other changes. The condition is in some respects a pyometra, except that the cervix is not closed, neither is the epithelium completely destroyed. The mucosa may be thrown into many extra folds which are thickened and oedematous. In one case a thick diphtheritic membrane covered the ventral surface of the uterine cavity and extended well up into both horns. This false inflammatory membrane was quite firmly united to the mucosa and underlying structure, the surface being rough and irregular with numerous projecting strands of fibrin.

The presence of sub-mucous cysts in large numbers has been frequently observed. They occur directly under the epithelial lining of the uterus and vary in size from approximately one millimeter to four or five centimeters in diameter. The contents consist of a clear amber-colored fluid. The cysts are usually well filled and extend above the general surface outline. They are not confined to this infection alone, as they are seen in metritis due to causes other than *Streptococcus genitalium* and have also been observed in uteri where infection was absent. A few uteri have been found that contained innumerable numbers of small upright, finger-like cysts just beneath the epithelium. All were from mares that were non-breeders, were bacteriologically negative during life, also upon post-mortem examination both from the surface epithelium and cyst content. The epithelium was apparently completely intact but had a moist, shiny, glistening appearance. The wall is thickened, soft, and spongy and when viewed from the mucous surface and particularly if moved or jarred slightly it has in general the appearance of a gelatinous mass. Occassionally large sub-epithelial cysts were found; it is assumed that they had formed from the union of numerous smaller cysts.

Pyometra. Many cases of chronic streptococcic metritis are in a degree pyometra. The exudate is strictly muco-purulent in character and the degenerated and eroded epithelium may be observed on gross examination. However, in that the cervical canal remains open, the pathological picture is somewhat different from that found in those cases where the canal becomes stenosed. Pyometra, then, as herein described, is confined to those cases where the exudate is retained in the uterine cavity because of complete closure of the cervical canal. A few such cases have been studied post-mortem. The uterus is distended, the wall thin, flaccid and atonic, and the corporeal cavity filled with a thick muco-purulent exudate. The epithelium is almost completely destroyed and the mucosa has become changed into a tough, thick, yellowish-white, fibrous pyogenic membrane. In one case of pyometra, due to streptococcic infection, the geni-

tals weighed 17½ pounds; after removal of the exudate the weight was 6½ pounds.

Microscopic sections of the uterus always show endometritis or inflammation of the endometrium is always seen. Metritis or inflammation of the muscularis occurs frequently, but probably always follows endometritis in this infection. Further evidence of this relation is shown by the fact that in cultures from infected uteri no growth has ever been obtained in media inoculated from areas beneath the mucosa.

In studying microscopic sections taken from the wall of the uterus it is essential to constantly keep in mind the normal cellular elements and their arrangement.

The normal uterine wall is divided into several rather distinct layers: The mucosa or endrometrium is composed of a single layer of columnar epithelial cells; the remainder, the tunica propria, is composed of an embryonal type of connective tissue rich in cellular elements. Many lymphocytes are normally present in this layer and occur in the vicinity of the lymphatics and smaller blood vessels. The submucosa is lacking. The muscularis is composed of circular and longitudinal muscle fibers. Three layers are recognized, however, in microscopic sections. Their outline is rather indistinct, and they appear as twisted bundles of muscle fibers held together by inter-muscular connective tissue. The serous coat or perimetrium consists of mesothelium, which rests upon a thin layer of connective tissue.

In studying sections taken from all parts of the uterine wall, including the horns, easily recognized lesions were observed. In general the lesions are similar to those involving the epithelium and underlying structures of the cervix. The most pronounced changes were edema of the mucosa, degeneration, desquamation, and sloughing of the epithelium. Infiltration of inflammatory round cells occurs frequently and is especially noticeable in and around those areas over which the epithelium has sloughed. They often penetrate the deeper structures and may be seen thruout the muscularis.

Exudation is especially noticeable over the denuded areas. The inflammatory product is composed of cell detritis, inflam-

matory round cells and mucus held together by fibrin and replaces the desquamated epithelium. The lumen of the uterine glands contains similar products which completely fill them in many instances.

A chronic productive inflammation in which there is a proliferation and fibrosis is the usual change seen in the muscular coats. The amount of this tissue formed is often several times more than is present in a normal uterus. Many inflammatory round cells are also seen thruout this area especially surrounding the blood vessels which are greatly distended with blood. Extensive hemorrhage is very common and is well diffused thruout the tissues. The edema so often seen in the mucosa is very seldom observed to penetrate this area.

The cases of pyometra examined show a chronic productive inflammation of the mucosa and the muscularis mucosa. The lining epithelium is replaced by a dense, thick, connective tissue and only traces of the uterine glands are found. The muscular coats contain a large amount of connective tissue and fibrosis was very evident thruout, together with round cell infiltration. Perimetritis is apparently very rare, as in no case of endometritis, metritis or pyometra did the serosa show noticeable patho logical change.

Salpingitis. Many cases show no pathological alterations in the fallopian tubes, others only a slight catarrhal inflammation characterized by a granular degeneration of the lining epithelium (in which the cells undergo degeneration, become swollen and enlarged and desquamate, allowing infiltration of inflammatory round cells into the deeper structures). In severe cases the entire wall is thickened with evidence of proliferation and fibrosis. Inflammatory products of a solid homogeneous mass that occlude the lumen are observed in only a few cases. Salpingitis, altho not common, is closely associated with endometritis and probably always occurs by ascending infection from the uterus.

Ovaritis. On the basis of cases studied, inflammation of the ovary is uncommon. What we recognize as senile changes were

noticed in many cases studied, characterized by atrophy and fibrosis. Nearly all the normal ovarian tissue was replaced by connective tissue which was very abundant thruout all sections examined. Cysts and follicles were usually found encased by a very thick dense fibrous tissue. No evidence of an acute inflammation was present in any of the post-mortem cases. Follicular and retention cysts apparently often result in an increase of connective tissue and in atrophy of the specific ovarian or germinal tissue. A number of greatly enlarged ovaries have been encountered on clinical examination, but were in mares not available for post-mortem. A few cases were successfully dealt with clinically, the mares were later bred, conceived and produced foals. In other cases the mares were sold under conditions that made it impossible to make further observations.

Two cases of sterility due to chronic ovarian trouble were killed and examined and will serve to illustrate how extensive some type of lesion in the ovary may become.

One is Case No. 1226 (see special report on page 224 of this bulletin). A second case, No. 7038, was that of a greatly enlarged ovary, Cut No. 1. The enlarged ovary was detected on clinical examination (Way), a tentative diagnosis of ovarian tumor was made and surgical removal was recommended. Some weeks later the ovary was found to have become so large as to make surgical removal impossible, hence the mare was destroyed. In shape the diseased ovary was a large circular flattened body measuring seven inches in its greatest diameter and four inches in thickness, had a dense fibrous capsule ¾ inch thick and was filled with a dirty reddish-brown necrotic purulent exudate containing large masses of coagula. Streptococcus genitalium was isolated from the exudate. The weight, after escape of a portion of the contained exudate thru an incision in the capsule, was 3½ pounds. Microscopic sections from the capsular wall showed a dense fibrous structure with inflammatory and proliferative changes on the inner surface. No definite tumor structure could be detected. Nevertheless it seems probable that the trouble may have started as a tumor, later becoming infected

and undergoing such complete degeneration as to obliterate all definite evidence of tumor structure.

Tumors. In the clinical examination of more than two thousand barren mares and the post-mortem examination of eighty-one mares, only six true tumor growths have been found in the reproductive organs; three ovarian tumors, two uterine tumors and one vaginal tumor. Two of the ovarian tumors

1. Left ovary. 2. Left fallopian tube. 3. Left horn. 4. Right horn. 5. Right fallopian. 6. Right ovary.

were diagnosed on clinical examination only. Both were located in the right ovary and were very hard and estimated as being about six inches in diameter. The third ovarian tumor (right ovary) was surgically removed and proved upon examination to be a typical epithelioma. The tumor was three and one-half inches in diameter, rather firm in consistency, and lobulated.

Of the two uterine tumors one was removed surgically. It was circular in outline, had a smooth surface and dense consistency and proved on examination to be typical fibroma. The other uterine tumor was a spreading, vascular growth on the

uterine mucosa. The mare was destroyed and upon examination the tumor was found to be of epithelial type but showed a great variety of structure.

The vaginal tumor was in an experimental mare killed for post-mortem examination and was examined and diagnosed as a fibro-sarcoma of benign type. When we consider the large number of mares examined, the small number of tumors found shows that tumors of the reproductive organs of mares are not of frequent occurrence.

Pathology of the Genital Tract in Cases of Infection With *Encapsulatus genitalium.*

Clinical Pathology. The presence of *Encapsulatus genitalium* in the genital tracts of mares results in the development of inflammatory processes that are clearly evident upon clinical examination. Externally, the hairs of the tail usually are soiled and matted, and dried exudate will be observed on the quarters adjacent to the external genitals. Upon rectal examination the walls of the uterus feel abnormally thick and the organ as a whole is enlarged. In those cases where there is an excessive accumulation of exudate, the uterus is distended in proportion to the amount of exudate present. In the case of old mares that have had several foals, and in which the uterus has become more or less dilated, pendulous and atonic, or in cases where adhesions have formed in the cervix, the exudate accumulates in the uterus in great quantities.

In a few cases the distension has been such that the condition might have been mistaken for early pregnancy; the uterus is, however, decidedly atonic, soft and doughy as compared with a physiological, gravid uterus. In young mares in which the uterus is well contracted and has retained its normal tone, the exudate which forms apparently is discharged from the uterus as fast as it forms. In general it has been our experience to find that the conformation of the cervix and uterus is not so apt to be distorted as in the case of cervicitis and metritis due to other types of infection.

The differential diagnosis of cervicitis and metritis in mares due to *Encapsulatus genitalium, Streptococcus genitalium* and other microorganisms should always be confirmed by a bacteriological examination. There are, however, certain changes rather typical and characteristic of this infection. First and most important of all is the character of the exudate. The exudate always is thick, often containing many flocculi, exceedingly viscid and tenacious in consistency and slimy to the touch. The color varies from a dull gray to a yellowish-white. The visible portion of the vaginal cervical membrane has a dull reddish-brown color.

During the period of estrus, exudation is profuse in all cases. In some cases, during the interval between the periods of estruation, the condition of the genital tract may return to a state approaching normal. However, by manual examination of the genital tract, the slimy exudate will be recognized and cultures are invariably positive, regardless of the time when they may be taken. The vaginal mucosa apparently is very resistant to this and other forms of bacteria. The secretion of exudate from the cervix and uterus, teeming with infection as it passes out over the vaginal mucous membrane, is naturally a source of great irritation, but does not result in any serious inflammatory processes. Upon control of the uterine secretion the vaginal membrane returns to normal.

Gross Pathology. The pathological changes are those of congestion, inflammation, exudation, degeneration, desquamation, and proliferation. The gross pathology of cases as they are met with in the field varies somewhat in the extent and character of the lesions. This variation is due in part to the condition of the genital tract of the mare at the time she becomes infected. In a number of cases studied the mares were suffering with metritis due to other causes before they became infected with *Encapsulatus genitalium.* In practically all such cases the lesions are found exaggerated over those observed in mares that were apparently normal when infection took place. Other conditions which tend to modify and intensify the lesions are age, length of time infection has been present, time of

estrual cycle, number and frequency of services during the breeding season, and, finally, the treatment that may have been employed. The inflammatory changes usually involve the entire genital tract; however, the main seat of pathological change is seen in the cervix and uterus.

The cervix is always involved in the inflammatory processes and contributes freely to the formation of exudate; however, the extent to which the cervix becomes distorted varies greatly in different cases, as stated under clinical pathology. The characteristic color of the vaginal cervix, as seen upon clinical examination, is a dark brownish-red; upon opening the cervical canal on post-mortem examination the color is found to be of a dark purplish-red. The cervical folds are thickened from inflammatory processes and covered with the characteristic slimy exudate. While the epithelial lining of the folds and of the excretory ducts and glands is always more or less involved, it is quite apparent that the muscular and fibrous structures do not always become seriously thickened thru inflammatory processes. In some cases the whole cervical structure has been found to be greatly enlarged. To know in just what proportion the enlargement is the result of infection with *Encapsulatus genitalium* or to causes operative previous to infection, would require that the condition of the genital tract of the mare be known previously to the time infection took place.

Upon opening the uterus the viscid exudate and dark purplish-red areas of discoloration stand out in great clearness as evidence of disease. The amount of exudate varies, depending upon the severity of the infection, but apparently more directly upon the condition of the uterus at the time infection took place. In what might be considered the average case, several hundred c. c. of the exudate will be found. The exudate is of a yellowish-white or dull-gray color, often very frothy and containing numerous flocculi and many cells of inflammatory and degenerative origin.

In the beginning, the process apparently is a typical endometritis. The whole surface of the uterine cavity is involved; the changes, however, are always more severe in the body of the

uterus than at the apices of the horns. The infection apparently remains more or less superficial, for while the walls of the uterus are thickened thru inflammatory processes, culture media inoculated from the deeper layers after removing the mucous membrane remain sterile.

Often there are irregularly-shaped hemorrhagic areas measuring from one to three centimeters in diameter, the outline being more or less easily traced. In cases of long standing the structures below the mucosa become involved thru chronic inflammatory processes, resulting in fibrosis, with a coresponding loss in and arrangement of the structures and tissues normally making up the various layers of the uterine wall.

In some uteri examined, numerous submucous cysts are found. Cysts of a similar character have been observed in other conditions and are therefore probably not a part of the pathology resulting from infection with encapsulated bacilli. Culture tubes inoculated from the cyst contents fail to show bacterial growth even in those cases in which microorganisms are present in the uterine exudate in great numbers.

Microscopic Pathology. Pathological changes found upon microscopic examination of sections of the uterus in the main substantiate the gross pathology. The sections examined were taken from several parts of each uterus. In each case the sections studied showed about the same degree of abnormality. This, as in the cases of *Streptococcus genitalium,* indicates that the inflammatory process is quite uniformly distributed thruout the uterus. Of all the different structures of the uterus the mucous membrane presents the most noticeable changes. Areas will be found in which the mucosa remains intact and shows only slight degeneration of the cells. The cells have a cloudy, granular appearance. The general outline of the cell may be lost and cannot be separated from its neighbor. Nuclei of the epithelial cells in these areas are seen in the lower part near the basement membrane; they also lose their distinctness of outline. In those areas where the epithelium is still intact, the submucosa, glandular and muscular layers are not severely involved.

In other cases, degeneration has progressed to such an extent that the mucosa is destroyed completely and sloughed. The exudate associated with these areas contains mucus, degenerated epithelium, cell detritus, pus cells and inflammatory round-cells in the reticular stroma immediately underlying the epithelium, especially in regions where there is a break in the continuity of the mucosa.

The deeper muscular layers which appear upon sections as twisted, irregularly-shaped bundles running in all directions may be still well preserved, but that they are later to become seriously involved in the inflammatory process is indicated by the presence of products of inflammation found scattered thruout all layers of the uterine wall.

The blood-vessels principally affected are those in the muscularis mucosa. They are greatly distended with blood and around each vessel is a zone of inflammatory round-cell infiltration. Another rather frequent condition existing is an edema in the submucous area.

In sections from cases of a more chronic nature the pathological picture is that of a typical chronic productive inflammation. Many areas will be found where the normal columnar epithelium of the mucosa has been replaced by a stratified squamous type.

The stratum nominally recognized as the submucosa and muscularis mucosa and in which the uterine glands are found, has become very largely obliterated during the course of the inflammation and is seen as a mass of newly-formed connective tissue. In the deeper muscular layers of the uterine wall the inflammatory process has been equally active. In some sections a typical granulation tissue is found. In others the inflammatory tissue is of a still more advanced type and has approached a state bordering on fibrosis. The newly-formed connective tissue penetrates the entire uterine wall from mucosa to serosa and has more or less completely displaced and obliterated the different layers and bundles of muscle tissue as normally observed in sections of the wall of the uterus.

Microscopic sections of the cervix show changes similar to those noticed in the uterus, except that they are, as a rule, more severe.

The surface epithelium and that lining the secretory glands undergo most severe inflammatory and degenerative changes, often resulting in complete destruction. The lumen of the glands may contain a thin mucus-like substance intermixed with cells and detritus or may be packed and distended with a solid, homogeneous mass. The opening of the gland ducts may be closed as a result of regeneration of the surface epithelium. The stroma is often involved in the early stages, showing changes of an acute inflammatory nature, later becoming enlarged and thickened in the form of a true hyperplasia.

Pyometra. Pyometra resulting from infection with *Encapsulatus genitalium* is in the main the same as that resulting from streptococcic infection. The most evident difference on gross examination is in the character of the exudate. In pyometra due to infection with *Encapsulatus genitalium* the exudate is of a whitish gray color and exceedingly tenacious and ropy in consistency. In one case there were two gallons of exudate in the uterus at autopsy. For approximately a period of one year the cervix had been completely closed by numerous adhesions.

Salpingitis. It has been our experience to find that inflammation of the oviducts is rare in mares. In a few cases a small amount of thick, yellow exudate has been found, rarely in quantity sufficient to occlude the tube completely. The successful passage of sperm cells would be doubtful, even in cases of mild inflammatory changes. In no case have the fallopian tubes been found enlarged, even in those cases where the infection had involved the tubes and ovaries. The pathological changes observed in the fallopian tubes have been limited mostly to a catarrhal salpingitis. In different sections of the same tube and sections of different tubes the changes noticed are quite uniform and mild in character. The lining epithelium shows a degeneration of a mild type. The cell bodies break down into a granular mass with poorly-staining nuclei. A mild infiltration by round cells sometimes is seen in the submucosa. The remaining mus-

cular layers and serosa appear normal. An exudate of mucus containing round cells and degenerated epithelium is often present in the lumen of the tube. Large, irregularly-shaped, homogeneous masses of uniform staining qualities are seen here quite often. This material is similar to that noticed in the cervical glands.

STREPTOCOCCUS GENITALIUM IN THE GENITAL TRACT OF MARES.

The mares listed in the Tables 1, 2 and 3 are not included in the averages given on page 160 because they are not primarily barren mares, altho a few in table 3 developed metritis before being bred, and others that were bred and failed to conceive later developed cervicitis and metritis. The purpose of the examinations made on the mares in Tables 1 and 2 was primarily to determine the natural habitat of *Streptococcus genitalium* and its relation to the genital tract of normal mares.

All mares in Table 1 had given birth to normal foals and otherwise foaled normally. With three exceptions the date of foaling is given. A comparison of the date of foaling and the first time cultured shows that the very great majority were cultured within the first week after foaling. Number 2704 was cultured 33 days after foaling, and No. 2688 was cultured 26 days after foaling. Those that were cultured a number of times are mares that were not bred that season or that were bred but failed to get in foal and were examined at a later date.

Table 2 gives a list of mares all but two of which were in foal at the time they were cultured. The examinations were made a few days or weeks before date of foaling. In taking the cultures, the lips of the vulva were spread, being careful not to touch the mucous membrane, the platinum needle was flamed, cooled, and the loop rubbed up and down on the mucous membrane of the lips of the vulva, about one-half inch in from the margin, and immediately transferred to a tube of agar slant medium, two tubes being inoculated from each mare.

Number 2803 was a maiden mare. After culturing the lips of the vulva a bacteriological examination was made of the cervix and uterus. The cultures made from the lips of the vulva were positive to streptococcus, and the cultures made from the

cervix and uterus were negative. Number 2137 was a non-infected mare that had been barren for two years. Culture tubes inoculated from the lips of the vulva were positive to streptococcus. Culture tubes inoculated from the cervix and uterus of this mare five times on different dates were all negative. This mare later was bred, conceived and gave birth to a normal foal.

In Table 3 are included mares that aborted and that were examined bacteriologically before being bred. The date the mare aborted and the date of the first examination are given showing the length of time that elapsed before culturing. All the mares in this list from which *Bacillus abortive-equinus* was recovered were from the same farm. There were approximately 100 brood mares, out of which a total of 40 aborted. Not all were examined, and not all those examined were positive on bacteriological examination to *Bacillus abortive-equinus;* however, all that were bled and the blood tested by the agglutination test for abortion were positive, including some that were not examined bacteriologically and including some of those examined bacteriologically but from which the cultures failed to show the abortion organism. Many of these mares developed metritis and were under treatment for a long time. This is indicated in part by the figures given in the column of times cultured.

In the case of ten of the mares on this list (Numbers 1865, 2270, 1288, 2590, 3094, 3157, 3180, 3189, 3190 and 3335), all of which aborted on the same farm, we were not able to recover *Bacillus abortivo-equinus* from the cervix and uterus. The tubes inocualted from five of the mares remained sterile, while streptococci developed on the tubes inoculated from the other five. The aborted fetuses from nine of these mares were submitted to a thoro bacteriological examination, with negative results for all. The mares had been vaccinated against contagious abortion and therefore we were not able to use the agglutination test to determine the absence or presence of *Bacillus abortivo-equinus.* Blood from all the mares was drawn and tested, and while agglutination was observed in sufficiently high dilutions to constitute a positive reaction, the reaction was prob-

ably the result of vaccination, since none of the reactions occurred in dilutions as high as occurs in abortion due to *Bacillus abortivo-equinus*. For example, No. 2517 aborted on March 7, 1924, was cultured March 13, 1924, cultures being positive to *Bacillus abortivo-equinus* and to streptococcus. Blood was collected on the date of abortion (March 7, 1924) and gave a reaction to abortion in dilutions 1-1000. In August, 1924, four months later, blood drawn from this mare still agglutinated to antigen of *Bacillus abortivo-equinus* in dilution of 1 to 1000. In the ten mares referred to above, in which the results of all bacteriological examinations made were negative, it is interesting to note that five were negative the first time cultured, and one was negative the second time cultured. The other four showed streptococcus on subsequent examination; some of them developed metritis and were under treatment for some time. These are indicated by the number of examinations noted in the column of times cultured.

TABLE 1—Foaling Mares.

No.	Date	Culture	Date	Culture	Date of Foaling	Times Cultured
956	3-25-24	Streptococcus	4-15-24	Negative	3-17-24	2
1005	4-15-24	Miscellaneous			4- 4-24	3
1214	4-22-24	Streptococcus			4-20-24	3
1318	5-20-22	Streptococcus			5-18-22	1
1467	4-15-24	Streptococcus			4-14-24	4
1477	2-25-24	Negative			2-18-24	2
1527	3- 7-24	Miscellaneous			3- 7-24	4
1530	3-25-24	Streptococcus	10-15-24	Negative	3-23-24	4
1628	2-28-24	Negative			2-24-24	4
1666	4-21-24	Negative			4-18-24	4
1779	3- 7-25	Miscellaneous			3- 4-25	5
1792	3-25-23	Negative			3-10-23	1
1795	3-24-23	Miscellaneous	3-31-23		3-18-24	1
2352	4- 8-25	Negative	10-29-25		4- 4-25	2
2397	1-31-24	Negative			1-31-24	1
2398	1-31-24	Negative			1-27-24	1
2431	2-15-24	Streptococcus			1-28-24	9
2497	3- 3-24	Miscellaneous	4- 3-24	Streptococcus	3- 3-24	11
2501	3- 7-24	Negative	4-30-24	Negative	2-29-24	2
2502	3- 7-24	Negative			3- 1-24	1
2537	3-14-24	Streptococcus			3- 7-24	1
2547	3-18-24	Rod	4-15-24	Negative	3-15-24	3
2548	3-18-24	Streptococcus	5- 1-24	Negative	3-14-24	3
2559	3-21-24	Miscellaneous			3-17-24	1
2582	3-25-24	Negative			3-23-24	1
2601-A	4- 5-24	Miscellaneous	4- 9-24	Miscellaneous	4- 1-24	4
2601-B	4- 5-24	Miscellaneous			3-29-24	1
2604	4- 5-24	Negative			3-29-24	1
2618	4-10-24	Streptococcus				2
2620	4- 9-24	Streptococcus			4- 4-24	1
2639	4-14-24	Streptococcus			4- 9-24	1
2640	4-14-24	Negative			4- 7-24	1
2645	4-15-24	Negative			4- 8-24	1
2684	4-22-24	Negative			4-18-24	1
2685	4-22-24	Negative			4-18-24	1
2688	4-24-24	Streptococcus			3-29-24	1
2691	4-24-24	Negative			4-19-24	1
2704	4-28-24	Negative			3-25-24	1
2732	4-15-24	Streptococcus			4-11-24	9
3213	1-21-25	Streptococcus			1-18-25	2
3357	3-28-25	Streptococcus	4- 1-25			2
3403	4- 8-25	Negative			4- 4-25	1
3424	4-15-25	Streptococcus			4-10-25	1
3449	4-26-25	Miscellaneous			4-21-25	1
3450	4-25-25	Streptococcus			4-23-25	3
3452	5- 7-25	Miscellaneous			5- 2-25	1
4790	5-10-26	Miscellaneous			4-27-26	1
4792	5-13-26	Streptococcus			5- 7-26	1
5064	4-19-26	Streptococcus			4-15-26	1
5155	5-18-26	Streptococcus	5-28-26	Negative		2
8307	2-16-27	Rods (Smear of vaginal exudate)			2- 8-27	1
8308	2-16-27	Rods (Smear of vaginal exudate)			2-10-27	1

TABLE 2—Cultures Taken from Lips of Vulva of Mares in Foal.

Number	Date	Culture	Times Cultured
1214	4-16-24	Streptococcus	1
1528	4-22-24	Streptococcus	1
1568	4-16-24	Streptococcus	1
1575	4-16-24	Streptococcus	1
1820	4-15-24	Streptococcus	1
2137	11-20-23	Streptococcus (barren)	1
2643	4-15-24	Streptococcus	1
2644	4-15-24	Miscellaneous	1
2666	4-16-24	Streptococcus	1
2667	4-16-24	Negative	1
2668	4-15-24	Streptococcus	1
2670	4-16-24	Negative	1
2671	4-16-24	Negative	1
2673	4-16-24	Negative	1
2803	6- 4-24	Streptococcus (Os negative, Maiden mare)	1
3426	4-22-25	Streptococcus, rod & micrococcus aureus	1
4935	2-24-26	Miscellaneous	1

TABLE 3—Abortion.

Number	Date	Culture	Date	Culture	Date of Slipping	Times Cultured
1288	1- 5-25	Negative	1-20-25	Negative	1-20-25	5
1481	10-10-22	Negative			10- 3-22	1
1600	2- 7-24	Strep. & B. abortivo-equinus	2-14-24	Streptococcus	2- 2-24	6
1628	12-21-23	Ps. pyocyanea			11-24-22	4
1769	4-16-24	Streptococcus			4-14-24	2
1865	1- 5-25	Streptococcus	1-20-25	Negative	1- 2-25	2
2215	11-14-23	Miscellaneous	11-20-23	Streptococcus	11-14-23	11
			12- 7-23	Negative		
2218	11-14-23	Negative			11- 8-23	1
2231	11-23-23	Negative		(Twins)	11-10-23	1
2270	12- 6-23	Streptococcus	12-17-23	Miscellaneous	12- 4-23	2
2333	12-24-23	Streptococcus			12-20-23	6
2405	2- 2-24	Streptococcus	2- 7-24	Strep. & B. abortivo-equinus	2- 2-24	6
2447	2-19-24	Miscellaneous	3-10-24	Negative	2-15-24	2
2496	3- 6-24	B. abortivo-equinus	3-11-24	B. abortivo-equinus & Strep. (twins)	3- 5-24	2
2515	3-13-24	Strep. & B. abortivo-equinus			3-10-24	1
2516	3-13-24	Strep. & B. abortivo-equinus		(Dead foal—full term.)	3-11-24	1
2517	3-13-24	B. abortivo-equinus & Strep.			3- 7-24	1
2518	3-13-24	Strep. & B. abortivo-equinus	5- 1-24	Streptococcus	3-11-24	2
2527	3-14-24	Miscellaneous			3- 5-24	1
2541	3-17-24	Streptococcus			3- 1-24	1
2542	3-17-24	Streptococcus			3-19-24	1
2544	3-17-24	Strep. & B. abortivo-equinus			3- 1-24	
2557	3-20-24	Streptococcus			3- 1-24	1
2590	4- 2-24	Negative			3-27-24	1
2591	4- 2-24	Negative			3-28-24	12

TABLE 3—Abortion—Continued.

Number	Date	Culture	Date	Culture	Date of Slipping	Times Cultured
2625	4-14-24	Strep. & B. abortivo-equinus			4-12-24	1
2626	4-14-24	B. abortivo equinus			4-14-24	1
2638	4-10-24	Strep. & B. abortivo-equinus			4- 8-24	1
3052	10- 2-26	Streptococcus	11- 8-24	Miscellaneous	10-12-26	1
3094	12- 1-24	Negative			11-13-24	1
3157	1- 5-25	Streptococcus			1- 2-25	10
3180	2- 6-25	Streptococcus			1-13-25	8
3189	1-22-25	Streptococcus			1-13-25	10
3190	1-15-25	Negative	1-22-25	Miscellaneous	1-15-25	1
3239	2- 4-25	Streptococcus			1-26-25	8
3255	2- 6-25	Miscellaneous	2-13-25	Miscellaneous	1-28-25	1
3335	3-17-25	Negative	4-21-25	Negative	3-13-25	1
3686	1-11-27	Miscellaneous			12-30-26	1
3941	11-16-26	Streptococcus & Ps. pyocyanea			10-18-26	2
4076	3-14-24	Streptococcus	4-17-24	Miscellaneous	1-28-25	5
4995	3- 5-26	Streptococcus			3- 2-26	1
9012	3-15-27	Streptococcus			3-11-27	1
9013	3-15-27	Streptococcus			2-15-27	1

The following table 4 gives a brief summary of conditions found at post-mortem. Nearly all the pathological changes noted were macroscopic. Microscopic sections were made, however, in nearly all instances and checked with those lesions which were found on gross examination.

Vaginitis, and especially cervicitis and metritis, were very common pathological conditions which were readily noticed at post-mortem. In this table no attempt was made to show the severity of infection in any case. All gradations from mild catarrhal inflammations to severe sloughing and degeneration were classified as one.

Salpingitis was uncommon. It appears to be the result of ascending infection from the uterus. Most tubes examined appeared normal upon gross examination. Those showing changes were characterized by enlargement due principally to the amount of exudate present. They contained a thick, yellowish, caseous exudate or a muco-purulent exudate which closed the tubes. These enlargements are usually seen at the ovarian end. All ovaries containing follicles are classified as normal. Cystic ovaries were interpreted as those which had abnormally large cysts and were known to be present over long periods of time as ascertained by clinical examination and verified upon post-mortem. A complete description of the clinical, macroscopic and microscopic pathology of infection is given under these separate headings in another part of this bulletin.

The bacteriological results given in the table were recorded for each mare examined at post-mortem irrespective of infection prior to death.

All results are tabulated under streptococcus, encapsulated bacilli, miscellaneous, and negative, according to the growth in the culture tubes.

TABLE 4.—Pathological and Bacteriological Findings in Mares Killed for Post-Mortem.

Condition of the Organs						Cultures from							
Mare No.	Vagina	Cervix	Uterus	Tubes	Ovaries	Cervix	Body Uterus	L. Horn	R. Horn	L. Tube	R. Tube	L. Ovary	R. Ovary
STREPTOCOCCUS FOUND													
957	Vaginitis	Cervicitis	Metritis	Normal	Normal	–	†	†	†	–	–	–	–
971	Normal	Cervicitis	Metritis	Normal	Normal	–	†	†	†	–	–	–	–
1479	Vaginitis	Cervicitis	Metritis	Normal	Normal	–	†	†	†	–	–	–	–
1483	Vaginitis	Cervicitis	Metritis	Salpingitis	Normal	†	–	–	–	–	–	–	–
1532	Vaginitis	Cervicitis	Metritis	Normal	Cystic	–	†	–	–	–	–	–	–
1572	Vaginitis	Cervicitis	Metritis	Salpingitis	Cystic	–	†	†	†	–	–	–	–
1761	Vaginitis	Cervicitis	Metritis	Normal	Cystic	°	†	†	†	°	°	°	°
1977	Vaginitis	Cervicitis	Metritis	Normal	Normal	–	†	†	†	–	–	–	–
2138	Vaginitis	Cervicitis	Metritis	Normal	Normal	†	–	–	†	–	–	–	–
2152	Vaginitis	Cervicitis	Metritis	Normal	Normal	°	†	†	†	–	–	–	–
2162	Vaginitis	Cervicitis	Metritis	Normal	Normal	†	†	†	†	–	–	–	–
2266	Normal	Cervicitis	Metritis	Normal	Normal	°	†	†	†	†	†	–	–
2287	Vaginitis	Cervicitis	Metritis	Normal	Normal	†	†	†	†	–	–	–	–
2288	Normal	Cervicitis	Metritis	Normal	Fibrosis	†	–	–	–	°	°	°	°
2498	Vaginitis	Cervicitis	Metritis	Normal	Normal	†	†	†	†	°	°	°	–
2734	Vaginitis	Cervicitis	Metritis	Normal	Normal	†	†	†	†	–	–	–	–
3030	Normal	Cervicitis	Metritis	Salpingitis	Normal	°	†	†	†	–	–	–	–
3049	Vaginitis	Cervicitis	Metritis	Salpingitis	Normal	–	†	†	†	–	–	–	–
3461	Vaginitis	Cervicitis	Metritis	Normal	Normal	–	†	†	†	–	–	–	–
4129*	Vaginitis	Cervicitis	Metritis	Normal	Cystic	†	†	†	†	–	†	–	–
4130*	Vaginitis	Cervicitis	Metritis	Salpingitis	Cystic	–	†	–	–	–	–	–	–
4799	Vaginitis	Cervicitis	Metritis	Salpingitis	Normal	†	†	–	–	–	–	–	–
5487*	Normal	Cervicitis	Metritis	Normal	Normal	†	–	–	–	–	–	–	–
5600*	Vaginitis	Cervicitis	Metritis	Salpingitis	Normal	†	†	–	–	–	–	–	–
6378	Normal	Cervicitis	Metritis	Normal	Normal	†	†	–	–	†	†	†	†
7038	Normal	Cervicitis	Metritis	Salpingitis	Ovaritis	°	°	°	°	°	°	°	†

TABLE 4.—Pathological and Bacteriological Findings in Mares Killed for Post-Mortem—Continued.

Mare No.	Vagina	Cervix	Uterus	Tubes	Ovaries	Cervix	Body Uterus	L. Horn	R. Horn	L. Tube	R. Tube	L Ovary	R. Ovary
ENCAPSULATED BACILLI FOUND													
1657*	Normal	Cervicitis	Metritis	Normal	Normal	+	+	+	+	—	—	—	+
1691	Vaginitis	Cervicitis	Metritis	Normal	Normal	+	+	+	+	—	—	—	+
2279	Vaginitis	Cervicitis	Metritis	Normal	Normal	—	+	+	+	—	—	—	—
2444	Vaginitis	Cervicitis	Metritis	Normal	Normal	+	+	+	+	+	+	+	+
2577	Vaginitis	Cervicitis	Metritis	Salpingitis	Normal	+	+	+	+	—	—	—	—
2720*	Normal	Cervicitis	Metritis	Normal	Normal	+	+	+	+	—	—	—	—
2798	Normal	Cervicitis	Metritis	Normal	Normal	+	+	—	—	—	+	+	+
2804	Vaginitis	Cervicitis	Metritis	Normal	Normal	+	+	+	+	—	—	—	—
3090	Vaginitis	Cervicitis	Metritis	Salpingitis	Normal	+	+	+	+	—	—	—	—
3284	Vaginitis	Cervicitis	Metritis	Normal	Normal	+	+	+	+	—	+	+	—
3654*	Normal	Cervicitis	Metritis	Normal	Normal	+	+	+	+	+	+	+	+
3952	Vaginitis	Cervicitis	Metritis	Normal	Cystic	+	—	+	+	—	—	—	—
4131*	Vaginitis	Cervicitis	Metritis	Salpingitis	Fibrosis	+	+	+	+	—	—	—	—
4132*	Vaginitis	Cervicitis	Metritis	Salpingitis	Fibrosis	+	+	+	+	—	—	—	—
4133*	Normal	Cervicitis	Metritis	Normal	Cystic	+	+	+	+	+	+	+	—
5599*	Normal	Cervicitis	Metritis	Normal	Cystic	+	—	+	+	+	+	+	—
5704*	Normal	Cervicitis	Metritis	Normal	Normal	+	+	+	+	+	+	+	+
6359*	Normal	Cervicitis	Metritis	Normal	Normal	+	+	+	+	+	+	+	+
MISCELLANEOUS BACTERIA													
717	Normal	Normal	Metritis	Normal	Normal	—	+	—	—	—	—	—	—
767	Normal	Cervicitis	Normal	Normal	Normal	—	—	—	—	+	+	—	—
768	Normal	Normal	Normal	Salpingitis	Fibrosis	—	—	—	—	—	+	—	+
799	Vaginitis	Cervicitis	Metritis	Salpingitis	Fibrosis	—	—	+	—	—	—	+	—
1006	Normal	Normal	Normal	Normal	Cystic	—	—	—	—	—	—	—	+
1041	Vaginitis	Cervicitis	Metritis	Normal	Fibrosis	—	—	+	—	—	—	—	—
1693	Normal	Normal	Normal	Normal	Normal	+	+	+	+	+	+	+	+
3374	Normal	Cervicitis	Metritis	Normal	Normal	+	+	+	+	+	+	+	+
5494	Normal	Cervicitis	Metritis	Normal	Normal	+	—	—	+	+	+	—	—

CULTURES NEGATIVE				
703	Normal	Cervicitis	Metritis	Normal	Normal
806	Normal	Normal	Normal	Normal	Fibrosis
841	Vaginitis	Normal	Normal	Normal	Cystic
893	Normal	Normal	Metritis	Salpingitis	Cystic
918	Vaginitis	Cervicitis	Metritis	Normal	Normal
936	Vaginitis	Cervicitis	Metritis	Salpingitis	Fibrosis
977	Normal	Normal	Metritis	Normal	Normal
1093	Normal	Normal	Normal	Normal	Fibrosis
1226	Normal	Normal	Normal	Normal	Fibrosis
1499	Normal	Normal	Metritis	Normal	Normal
1515	Normal	Cervicitis	Metritis	Normal	Normal
1526	Normal	Normal	Metritis	Normal	Small
1533	Normal	Normal	Normal	Normal	Normal
1560	Vaginitis	Cervicitis	Metritis	Salpingitis	Fibrosis
1655	Normal	Normal	Normal	Normal	Normal
2104	Normal	Normal	Normal	Normal	Normal
3048	Vaginitis	Cervicitis	Metritis	Normal	Normal
3283	Normal	Normal	Normal	Normal	Normal
3600	Vaginitis	Cervicitis	Metritis	Normal	Normal
3653*	Vaginitis	Cervicitis	Metritis	Salpingitis	Normal
3689	Normal	Cervicitis	Metritis	Normal	Normal
3925	Normal	Normal	Normal	Normal	Normal
5213*	Vaginitis	Cervicitis	Metritis	Normal	Normal
5214*	Normal	Cervicitis	Metritis	Normal	Fibrosis
5215*	Normal	Cervicitis	Metritis	Normal	Normal
5602	Vaginitis	Cervicitis	Metritis	Normal	Normal
7408	Vaginitis	Cervicitis	Metritis	Normal	Normal

- Culture tubes inoculated yielded no growth.

† Culture tubes inoculated yielded positive cultures.

° No culture tubes inoculated.

* Experimental animal.

Cultural Characteristics of *Streptococcus genitalium*

The isolation of this organism is accomplished without great difficulty, since beef infusion agar, the reaction of which is pH 7.2-7.4, fosters a satisfactory growth.

The growth of the primary culture is slightly more luxuriant than that of the subsequent transfers, due perhaps to the presence of body fluids in the primary culture. The addition of fresh, sterile horse serum to the medium seems to favor growth. The optimum temperature of the organism is 37° C, the development at room temperature being slow.

The organism is a Gram positive streptococcus, occurring in short chains in the exudate from the uterus and solid media. In liquid media the chains are made up of a large number of elements and may be long and tangled. As is usual with streptococci, the organisms vary somewhat in size, large and small cocci occurring in the same chain. It stains readily with the ordinary anilin dyes.

On agar, *Streptococcus genitalium* produces a delicate growth, with a marked tendency to colonize. Agar colonies vary in size from a pin point to one mm in diameter. The larger colonies have a slightly milky appearance, while the smaller colonies are colorless.

In beef infusion broth, pH 7.2-7.4, a fairly abundant growth is obtained within 24 hours. The majority of the cultures isolated present a granular growth in broth, with a clear supernatant fluid. Some cultures, however, produce an even clouding of the liquid with very little sediment. The character of the growth in liquid media is influenced largely by the hydrogen ion concentration.

In fresh blood media, *Streptococcus genitalium* produces a rapid hemolysis, belonging to the beta streptococci, according to the classification of Brown.*

Milk is slowly acidified but rarely coagulated. Dextrose, lactose, sucrose and salicin are readily fermented, while mannitol, raffinose and inulin are not attacked.

*Brown, G. H., Monograph 19, Rockefeller Inst. for Med. Res.

The organism is readily destroyed by heat or sunlight and survives only 14 days on the surface of an agar slant under the most favorable conditions.

Pathogenicity Tests

Streptococcus genitalium is pathogenic for rabbits, but non-pathogenic for rats and guinea pigs. Subcutaneous inoculations in rats, and subcutaneous, intraperitoneal and intravenous inoculations, together with feeding experiments on guinea pigs, failed to produce any ill effects.

One-fourth cubic centimeter (approximately five billion organisms) of virulent strain, recently passed thru an animal, injected intravenously, will kill a large rabbit in from twelve to thirty-six hours.

One cubic centimeter of the same culture, injected subcutaneously into a rabbit does not kill, while the same amount given intraperitoneally kills in from four to five days, and when fed, causes death in a week to ten days.

By accident a healthy rabbit ate of a rabbit that had just died from an injection of the streptococcus, and in twelve hours the animal died and the organism was recovered from its blood. This, of course, was a particularly virulent strain, as it had just been passed thru several animals and was ingested without having been on artificial media since its passage thru an animal.

Characters of *Encapsulatus genitalium*

Morphology and Staining

This bacterium is a non-motile, encapsulated rod. The morphology of the organism is variable. It varies in length from .9 to 1.7 microns. Long rods and coccoid forms may be encountered in the same smear. Especially is this variability in size and form noticeable in young cultures. In older cultures the organisms tend to assume a coccoid form.

The bacterium is Gram negative and does not produce spores. It is stained readily by the ordinary anilin dyes. The capsules are easily demonstrated and are unusually large. This

heavy capsule is responsible for an excessively mucoid form of growth which is the outstanding cultural characteristic on all media.

Cultural Characteristics

On agar slants there occurs an excessive dirty white to slightly yellow growth which is moist, spreading, glistening and very viscid. Agar colonies are large, raised, round and entire. Broth becomes turpid, with a ropy sediment. A typical nailhead growth appears in gelatin; no liquefaction occurs. Acid and coagulation are produced in litmus milk. Nitrates are reduced to nitrites and ammonia. Indol is not formed.

Comparative Bacteriological Studies of Encapsulated Bacilli

The above characteristics place the bacterium in the genus *Encapsulatus*. Included in this genus are several species. The best known of these is *Encapsulatus pneumoniae* or Friedlander's bacillus. This organism was originally advanced as the causative agent of pneumonia in man. Since that time cultures morphologically and culturally similar to this bacterium have been isolated from a variety of sources. These cultures have been designated by many investigators as *B pneumoniae* or Friedlander's bacillus. Consequently at present we have many cultures isolated from diverse sources which are included in this species. The name has been used as a designation for encapsulated bacilli, regardless of their characters or the sources from which they were isolated.

Other members of this genus are:

Encapsulatus inguinalis, found associated with the local lesions of inguinal granuloma.

Encapsulatus rhinoscleromatis, isolated from the nasal discharge of patients affected with rhinoscleroma.

Encapsulatus ozenae, isolated from cases of ozena.

In addition to these species included in the genus Encapsulatus there are many strains of *Bact. aerogenes* (now classi-

fied in the genus Aerobacter) which present similar cultural characters.

Many investigators have worked with these organisms, but little order has been established in the group until the recent work of Small and Julianelle (1) and Julianelle (2, 3, 4).

Since the encapsulated organisms recovered from mares were so closely related to the other members of the group it was thought best to make a comparative study of several members of the Encapsulatus group. For this purpose 25 strains of equine origin were isolated and carried as stock cultures. Strains of Friedlander's bacillus *B. granulomatis*, *B. rhinoscleromatis* and encapsulated strains of *Bact. aerogenes* were obtained from different laboratories and with these organisms comparative studies were carried out.

Following is a list of the strains, with the symbols by which they were designated, their source, the clinical diagnosis of the cases from which they were isolated and, when known, the date of isolation.

Strain No.	No.	Clinical Diagnosis	Source	Date Isolated
E 1	1459	Metritis	Uterus	
E 2	3621	Metritis	Uterus	
E 3	5093	Metritis	Uterus	5- 6-26
E 4	3849	Metritis	Uterus	11-30-25
E 5	3952	Metritis	Uterus	12-16-25
E 6	2390	Metritis	Uterus	4- 2-26
E 7	3758	Metritis	Utreus	10-29-26
E 8	4008	Metritis	Uterus	1- 6-26
E 9	5242	Metritis	Uterus	8-11-26
E 10	2333	Metritis	Uterus	11-30-25
E 11	2305	Metritis	Uterus	4-19-26
E 12	2928	Metritis	Uterus	Spring 1925
E 13	5087	Metritis	Uterus	5- 6-26
E 14	5098	Metritis	Uterus	5- 6-26
E 15	3838	Metritis	Uterus	11-30-25
E 16	5094	Metritis	Uterus	5- 6-26
E 17	5092	Metritis	Uterus	5- 6-26
E 18	4006	Metritis	Uterus	1- 6-26
E 19	2927	Metritis	Uterus	11- 9-25
E 20	2445	Metritis	Uterus	11- 9-25
E 21	5097	Metritis	Uterus	5- 6-26
E 22	2444	Metritis	Uterus	9-11-25
E 23	3811	Metritis	Uterus	11-19-25
E 24	2359	Metritis	Uterus	11-15-26
E 25	4799	Metritis	Uterus	2-23-26

List of Strains—Continued.

Strain No.	Clinical Diagnosis	Source	Isolated
G 2	Granuloma inguinale	Local lesion	3/8/21
G 3	Granuloma inguinale	Local lesion	4/27/21
G 4	Granuloma inguinale	Local lesion	9/20/21
G 5	Granuloma inguinale	Local lesion	10/29/21
G 8	Granuloma inguinale	Local lesion	11/28/21
G 9	Granuloma inguinale	Local lesion	1/2/22
G 10	Granuloma inguinale	Local lesion	1/21/22
G 11	Granuloma inguinale	Local lesion	5/5/22
G 13	Granuloma inguinale	Local lesion	8/8/22
G 14	Granuloma inguinale	Local lesion	2/18/23
F 2		Extirp. tonsil	3/3/21
F 3	Pneumonia	Heart blood	5/6/21
F 5	Pneumonia	Sputum	5/6/21
F 8	Pneumonia	Heart blood	7/3/21
F 9	Pneumonia	Sputum	8/26/21
F 10	Pneumonia	Lung tissue	(?)
F 11	Pneumonia	Heart blood	12/14/21
F 12	Pellagra	Feces	12/14/21
F 13	Pneumonia	Sputum	3/17/22
F 15	Mitral regurgitation	Sputum	4/9/22
F 18	Pneumonia	Sputum	(?)
F 19	Pleuritis	Blood in life	(?)
F 20	Pneumonia	Heart blood	(?)
F 21	Appendicitis	Surgical extirp. appendix	6/7/23
F 22	Pneumonia	Heart blood	6/1/23
F 23	Pneumonia	Blood in life	7/8/23
F 24	Pneumonia	Sputum	8/19/25
F 25	Pneumonia	Sputum	8/24/25
A 1	Stock culture Bact. aerogenes	From feces	

A 3	Stock culture Bact. aerogenes	From soil	
A 11	Stock culture Bact. aerogenes	B. aerogenes	
A 12	Stock culture Bact. aerogenes	B. aerogenes	
R 1	Stock culture Bact. aerogenes	B. rhinoscleromatis case of rhinoscleroma	(?)
R 11	From nasal cult.		
EP V	Granuloma inguinale	Local lesion	6/3/21
M3B1	Granuloma inguinale	Local lesion	5/9/21
F 26	Chronic colitis	Feces	
F 27	Chr. cystitis	Urine	
F 28	Pneumonia c { Lung abscess / Empyemia }	{ Sputum	
FHM	Pneumonia	Sputum	7/15/26
G 6	Granuloma inguinale	Local lesion	(?)
F 1			
F 6	Pneumonia	Sputum	(?)
S c	Pneumonia	Heart blood	(?)
FR	Stock culture. Friedlander's bacillus	History unknown	

Biochemical Studies

The cultures of encapsulated bacilli were subjected to the following biochemical tests: Indol production, growth in citrate and uric acid media, nitrate reduction, production of ammonia, action in litmus milk, production of acetyl-methyl carbinol, methyl-red test, and the production of acid and gas from a number of fermentable substances. A summary of the reactions of the various strains to the tests is given in Table 9.

TABLE NO. 5—Biochemical Reactions.

	Citrate	Indol	Methyl Red	Voges-Proskauer	Uric Acid	Litmus Milk	Nitrate Reduction	Ammonia Production
E 1	+	—	—	+	+	AC	+	+
E 2	+	—	—	—	+	AC	+	+
E 3	+	—	—	+	+	AC	+	+
E 4	+	—	—	+	+	AC	+	+
E 5	+	—	—	+	+	AC	+	+
E 6	+	—	—	+	+	AC	+	+
E 7	+	—	—	—	+	AC	+	+
E 8	+	—	—	+	+	AC	+	+
E 9	+	—	—	+	+	AC	+	+
E 10	+	—	—	+	+	AC	+	+
E 11	+	—	—	+	+	AC	+	+
E 12	+	—	—	+	+	AC	+	+
E 13	+	—	—	+	+	AC	+	+
E 14	+	—	—	+	+	AC	+	+
E 15	+	—	—	—	+	AC	+	+
E 16	+	—	—	+	+	AC	+	+
E 17	+	—	—	+	+	AC	+	+
E 18	+	—	—	+	+	AC	+	+
E 19	+	—	—	+	+	AC	+	+
E 20	+	—	—	+	+	AC	+	+
E 21	+	—	—	+	+	AC	+	+
E 22	+	—	—	+	+	AC	+	+
E 23	+	—	—	+	+	AC	+	+
E 24	+	—	—	+	+	AC	+	+
E 25	+	—	—	+	+	AC	+	+
F 1	+	—	+	—	—	A	+	+
F 2	+	—	+	—	+	A	+	+
F 3	+	—	—	+	+	A	+	+
F 5	+	—	—	+	+	A	+	+
F 6	+	—	—	+	—	AC	+	+
F 8	+	—	—	—	+	A	+	+
F 9	+	—	—	+	+	A	+	+
F 10	+	—	+	—	+	A	+	+
F 11	+	—	—	+	+	AC	+	+

TABLE NO. 5—Biochemical Reactions—Continued.

	Citrate	Indol	Methyl Red	Voges-Proskauer	Uric Acid	Litmus Milk	Nitrate Reduction	Ammonia Production
F 12	—	—	+	—	+	AC	+	+
F 13	—	—	+	—	—	A	+	+
F 15	+	—	—	+	+	A	+	+
F 19	+	—	—	+	+	A	+	+
F 20	—	—	—	+	—	A	+	+
F 21	+	—	+	—	+	AC	+	+
F 22	—	—	—	—	—	—	+	+
F 23	+	—	—	+	+	A	+	+
F 24	+	—	+	—	+	A	+	+
F 25	+	—	—	+	+	A	+	+
F 26	+	—	—	+	+	AC	+	+
F 27	+	—	—	+	+	A	+	+
F 28	+	—	—	+	+	A	+	+
Sc	+	—	+	—	+	A	+	+
F 18	+	—	—	+	+	A	+	+
FR	+	—	—	+	+	A	+	+
G 2	+	—	—	—	+	AC	+	+
G 3	—	—	+	—	+	AC	+	+
G 4	—	+	+	—	+	AC	+	+
G 5	—	+	+	—	+	AC	+	+
G 6	+	—	—	+	+	AC	+	+
G 8	+	—	—	+	+	AC	+	+
G 9	—	—	—	—	—	—	+	+
G 10	—	—	—	—	—	—	+	+
G 11	+	—	—	—	—	AC	+	+
G 12	—	—	—	—	—	—	+	+
G 13	+	—	—	—	+	AC	+	+
G 14	—	—	—	—	—	AC	+	+
G 15	—	—	—	—	—	—	+	+
G 16	+	—	—	+	—	—	+	+
EPV	—	—	—	—	—	—	+	+
M3B1	—	—	—	—	—	—	+	+
R 1	—	—	—	—	—	—	+	+
R 2	+	—	+	—	+	A	+	+
A 1	+	—	—	+	+	AC	+	+
A 3	—	—	—	+	+	A	+	+
A 11	+	—	—	+	+	A	+	+
A 12	—	—	+	—	+	A	+	+

A—Acid production.
AC—Acid production and coagulation.

From the results noted in Table 5 it appears that the 25 strains recovered from mares are exceedingly uniform. All the equine cultures failed to produce indol, were able to initiate

CULTURES OF ENCAPSULATUS GENITALIUM OBTAINED ON POST-MORTEM EXAMINATION

1. Culture tubes inoculated from cervix. 2. Culture tubes inoculated from body of uterus. 3. Culture tubes inoculated from left horn. 4. Culture tubes inoculated from right horn.

growth in uric acid and citrate media, reduced nitrate to nitrites, produced ammonia, caused acid and coagulation in litmus milk, were Voges-Proskauer positive and methyl-red negative.

In the Friedlander and granuloma groups no such uniformity was demonstrable. None of these cultures produced indol, all reduced nitrate and produced ammonia. Their reactions to the other tests varied widely.

Twenty-two of the Friedlander strains were able to initiate growth in citrate medium; only seven granuloma vultures were able to develop in this medium. Nine of the Friedlander strains were positive and eighteen negative to methyl-red. Sixteen of the Friedlander cultures gave positive Voges-Proskauer tests and eleven were negative. Of the granuloma cultures, three were methyl-red positive and fifteen were negative, while two gave positive Voges-Proskauer tests, and sixteen were negative. Among the Friedlander bacilli there is a fairly close correlation between the methyl-red and Voges-Proskauer tests. No such correlation is demonstrable among the granuloma strains. The action of the Friedlander and granuloma organisms in litmus milk was quite varied.

The most striking facts brought out in Table 5 are the unusual uniformity of the equine strains and their reactions to these tests. In every particular they give reactions which have been considered typical of the soil types of *Bact. aerogenes.*

Fermentation Reactions

All the cultures were tested for their ability to produce acid and gas from the following fermentable substances: Dextrose, levulose, galactose, lactose, sucrose, glycerol, dulcitol, mannitol, sorbitol, arabinose, xylose, raffinose, maltose, adonitol, salicin, inulin, dextrin, and soluble starch. The medium used to test for fermentation was meat extract broth containing 1% Andrade's indicator. Durham fermentation tubes were used to observe gas formation. The fermentable substances were sterilized by filtration thru Berkefeld filters and added to the previously sterilized broth in sufficient amount to give a final con-

centration of 1%. Observations were recorded at 24 hours, 48 hours, and 10 days after inoculation. The results of these tests are given in Table 6.

TABLE NO. 6—Fermentative Reactions.

	Dextrose	Levulose	Galactose	Lactose	Sucrose	Glycerol	Dulcitol	Mannitol	Sorbitol	Arabinose	Xylose	Raffinose	Maltose	Adonitol	Salicin	Inulin	Dextrin	Starch
E 1	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
E 2	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
E 3	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
E 4	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
E 5	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
E 6	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
E 7	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
E 8	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
E 9	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
E 10	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
E 11	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
E 12	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
E 13	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
E 14	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
E 15	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
E 16	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
E 17	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
E 18	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
E 19	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
E 20	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
E 21	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
E 22	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
E 23	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
E 24	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
E 25	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
F 1	A	A	A	A	O	A	O	A	A	A	A	A	A	A	A	O	A	A
F 2	AG	AG	AG	AG	O	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG

TABLE NO. 6—Fermentative Reactions—Continued.

	Dextrose	Levulose	Galactose	Lactose	Sucrose	Glycerol	Dulcitol	Mannitol	Sorbitol	Arabinose	Xylose	Raffinose	Maltose	Adonitol	Salicin	Inulni	Dextrin	Starch
F 3	AG	AG	AG	AG	AG	O	O	AG	AG	A	AG	AG	AG	AG	AG	O	AG	AG
F 5	AG	AG	AG	A	AG	O	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
F 6	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
F 8	AG	AG	AG	AG	AG	O	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
F 9	AG	AG	AG	AG	AG	O	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
F 10	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
F 11	A	A	A	O	A	O	O	A	O	A	A	O	O	O	O	O	O	O
F 12	AG	AG	AG	AG	O	AG	O	AG	AG	AG	AG	AG	AG	O	O	O	AG	A
F 13	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
F 15	AG	AG	AG	AG	AG	A	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
F 18	A	A	A	A	A	A	O	A	A	A	A	A	A	A	A	O	A	A
F 19	AG	AG	AG	AG	AG	O	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
F 20	AG	AG	AG	AG	A	O	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
F 21	AG	AG	AG	A	AG	A	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
F 22	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
F 23	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
F 24	AG	AG	AG	AG	AG	O	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
F 25	AG	AG	AG	AG	AG	O	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
F 26	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
F 27	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
F 28	AG	AG	AG	AG	AG	O	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
Sc	A	A	A	A	A	A	A	AG	AG	A	A	A	A	A	A	O	AG	AG
G 2	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG
G 3	AG	AG	AG	AG	O	O	O	AG	AG	AG	AG	O	AG	O	O	O	A	A
G 4	AG	AG	AG	AG	O	AG	O	AG	AG	AG	AG	O	AG	O	AG	O	AG	O
G 5	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	O	O	AG	O	O	O	AG	O
G 6	A	AG	A	AG	A	A	O	A	A	A	A	A	A	AG	A	O	AG	AG

G 8	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG
G 9	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
G 10	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
G 11	A	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
G 13	A	A	O	O	O	O	O	A	A	O	O	O	O	O	O	O	O	O
G 14	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
EPV	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
M3 B1	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
R 1	A	A	A	A	A	O	A	A	A	A	A	A	A	O	A	A	A	A
R 2	A	A	A	AG	AG	O	AG	AG	AG	AG	AG	A	AG	O	A	AG	AG	AG
A 11	A	A	A	A	A	A	AG	A	A	A	A	A	AG	O	A	AG	A	A
A 12	A	AG	AG	AG	O	O	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	O
A 3	AG	AG	AG	AG	AG	O	AG	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG
A 1	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	AG

A—Acid formation without gas.
AG—Acid and gas formation.
O—Neither acid nor gas formation observed.

From Table 6 it is evident that the equine strains are identical in their action on the substances tested. Acid and gas were produced by all strains from dextrose, levulose, galactose, lactose, sucrose, glycerol, mannitol, sorbitol, arabinose, xylose, raffinose, maltose, adonitol, salicin, dextrin and soluble starch. Dulcitol and inulin were not attacked.

The granuloma and Friedlander bacilli varied markedly in their action on the fermentable substances used in the work. Some of the strains were very active fermenters, while others were apparently non-saccharolytic. Some of the strains produced acid without gas formation from any of the sugars. Other strains produced acid in the majority of the sugars while producing gas in only one or two. The results confirm the work of Fitzgerald (5) and Small and Julianelle (1). These workers demonstrated that fermentation in the mucosus group was highly variable and was not a reliable criterion for the purpose of classification.

One fact is noteworthy, the production of gas by R2, isolated from a case of rhinoscleroma. Rhinoscleroma strains have generally been reported as not forming gas. From Table 6 it can be seen that strain R2 produced gas from the majority of the substances tested. This gas production occurred only after four or five days' incubation and usually the amount produced was small, not exceeding 10% to 15%. The only other rhinoschleroma strain studied, R1, was never observed to produce gas.

Serological Reactions

Agglutination Tests: Many workers have attempted to classify the encapsulated organisms by serological methods, but they have met with little success. It has been found more or less difficult to prepare agglutinating sera. The physical nature of the capsular substance has been thought to be responsible for this difficulty. Several methods have been devised by different workers to overcome this condition. Porges (6) hydrolyzed the capsule by heating with acid. Beham (7) used artificial selection to avoid the inhibitory effects of the capsules. Under these conditions agglutination was obtained regularly with Fried-

lander bacilli. Coulter (8) had difficulty in preparing agglutinating sera for Friedlander's bacilli, but found them of uniform character serologically, regardless of source. Small and Julianelle (1), working with Friedlander's and granuloma bacilli, identified serologic groups but could not arrive at a satisfactory practical classification. Toennissen (9) advanced the idea of distinct capsular and endoplasmic antigens in the mucosus group. Julianelle (2, 3, 4) demonstrated that type specific antigen in this group was associated with the carbohydrate of the capsule, while the nucleoprotein of the endoplasm was only species specific. He studied thirty Friedlander's cultures and divided them into four types on the basis of serological tests.

In the present work agglutinating sera have been prepared for several of the strains being studied. Rabbits were employed in the preparation of these sera.

In preparing the agglutinating antigens 24-hour agar cultures were suspended in carbolized saline and the turbidity of the suspension adjusted to 0.5 on the McFarland nephelometer scale. The agglutination tests were incubated 48 hours at 37° before being read.

The results of the agglutination tests are given in Table 7.

TABLE NO. 7—Agglutination Reactions.

Antigens	Serum E 1	Serum G 2	Serum G 6	Serum F 6	Serum Sc	Serum Normal
E 1	500	0	0	200	0	0
E 2	500	0	0	200	0	0
E 3	500	0	0	200	0	0
E 4	500	0	0	200	0	0
E 5	1000	0	0	200	0	0
E 6	1000	0	0	200	0	0
E 7	500	0	0	200	0	0
E 8	200	0	0	200	0	0
E 9	200	0	0	200	0	0
E 10	200	0	0	100	0	0
E 11	200	0	0	200	0	0
E 12	500	0	0	100	0	0
E 13	200	0	0	50	0	0
E 14	200	0	0	50	0	0
E 15	500	0	0	200	0	0
E 16	200	0	0	50	0	0
E 17	200	0	0	100	0	0
E 18	200	0	0	50	0	0
E 19	500	0	0	200	0	0
E 20	500	0	0	100	0	0
E 21	100	0	0	50	0	0
E 22	200	0	0	50	0	0
E 23	500	0	0	200	0	0
E 24	500	0	0	200	0	0
E 25	500	0	0	200	0	0
F 1	0	0	0	0	0	0
F 2	0	0	0	0	0	0
F 3	0	0	0	0	50	0
F 5	0	0	0	0	100	0
F 6	200	0	0	200	0	0
F 8	0	0	0	0	100	0
F 9	0	0	0	0	20	0
F 10	0	0	0	0	0	0
F 11	0	0	0	0	20	0
F 12	0	0	0	0	0	0
F 13	0	0	0	0	0	0
F 15	0	0	0	0	50	0
F 18	0	0	0	0	50	0
F 19	100	100	500	500	1000	100
F 20	0	0	0	0	50	0
F 21	0	0	0	0	50	0
F 22	0	0	0	0	0	0
F 23	0	50	200	20	1000	100
FR	200	0	0	100	0	0
A 3	500	0	0	200	0	0
F 24	0	0	0	0	20	0
F 25	0	0	0	0	50	0
F 26	200	0	0	100	0	0
R 1	0	20	20	20	20	20
Sc	0	0	0	0	100	0
F 27	0	0	0	0	0	0
F 28	0	0	0	0	55	0

0 Indicates no agglutination at a dilution of 1-20.
Numbers indicate highest dilution in which agglutination occurred.

The results of the agglutination tests are comparable to the results obtained in the biochemical reactions. The equine strains all belong to a single agglutinative type. It is extremely unusual for such a large group of encapsulated bacilli, all recovered from the same source, to fall into a single type. These twenty-five cultures recovered from mares are not a selected group of strains but were collected just as they came into the laboratory without examination of their biochemical or antigenic character. In addition to these twenty-five cultures some fifteen additional strains have been tested and found to belong to the same type. This gives a total of forty cultures recovered from cases of cervicitis and metritis in mares, all belonging to the same agglutinative type. We have never encountered an encapsulated bacillus from a case of metritis which did not belong to this type. Such a large number of encapsulated bacilli from a single source, all of which belong to the same type, has never before been reported. Encapsulated bacilli from a single source usually are divisible into two or more types on the basis of serological tests.

Two of the Friedlander cultures, F6 and F26, belong to the same agglutinative type as do the equine strains. One culture of *Bact. aerogenes,* A3, also falls into this group. A second large group is established in the Friedlander strains by the agglutination test. Sixteen of the strains were agglutinated from the serum derived from culture Sc. These strains were isolated from a variety of sources, the majority being found associated with pneumonia.

Seven of the Friedlander strains could not be typed in either of these groups. Some of these untyped strains were no longer producing a heavy capsule so that typical agglutination did not occur. Whether these strains are antigenically independent cannot be stated, since serum has not been prepared from them.

These results confirm the work of Julianelle (2). The Friedlander strains employed in this study are largely the same as those used by him and the groupings have, with one or two exceptions, been the same.

Precipitin Tests: Precipitin tests have been performed and the results confirm those of the agglutination tests, the groupings being the same by both methods.

Agglutinin Absorption Tests: Agglutinin absorption tests have also been performed. The absorption tests have shown the equine cultures to be agglutinatively identical. Also the strains giving type specific agglutination with serum Sc have been found to completely absorb agglutinins from this serum.

Cultures F6, F26 and A3 exhaust the agglutinins from sera derived from the equine strains.

Pathogenicity

The cultures of encapsulated bacilli recovered from mares are pathogenic for mice, rats, guinea pigs and rabbits. When one cubic centimeter of a 24-hour broth culture is injected subcutaneously death follows within twenty-four hours. Toennissen (10) and Julianelle (2, 3, 4) have demonstrated that the virulence of Friedlander's bacillus is dependent on capsule formation. The capsulated strains are of uniformly high virulence, while strains which have lost the property of producing capsules are non-virulent. The same conditions have been found to exist in this group of bacteria. Cultures which have been under artificial cultivation for some time and which have lost their property of producing heavy capsules and their mucoid form of growth, are no longer virulent for the laboratory animals when injected subcutaneously.

Virulence can be enhanced by reviving capsule formation. This may be accomplished by animal passage if the organism has not completely lost its power to produce death in the laboratory animals when injected in large amounts. Artificial selection may also be used to increase the virulence of cultures which have partially lost their pathogenicity. By successive platings on glucose agar and picking those colonies which exhibit the most mucoid, tenacious form of growth, capsule formation may often be revived. In this way cultures may often be brought back to full capsule formation and virulence.

Infection Experiments

Dimock and Edwards (11) reported the reproduction of the type of cervicitis and metritis found in field cases in mares by intra-uterine inoculation with pure cultures of encapsulated bacilli recovered from these conditions. At this time it was reported that Friedlander's bacillus did not cause cervicitis and metritis when inoculated intrauterally. Since that time a number of additional strains of Friedlander's bacillus have been used in an effort to reproduce the disease. Certain strains of Friedlander's and one strain of *Bact. aerogenes* have been found to reproduce the condition.

Within a few days after inoculation with encapsulated bacilli capable of causing disease, the mares develop cervicitis and metritis with a copious, slimy exudate containing flocculi, leucocytes and tissue detritus. The organisms may be recovered in great numbers by culturing the os uteri. When organisms which are incapable of bringing about this condition are introduced into the uterus the results are quite different. Even tho the whole of an agar slant culture is placed in the uterus the mares show no clinical evidence of infection. The organisms which have been introduced disappear rapidly. If the os uteri is cultured 48 to 72 hours after inoculation, the organisms cannot be recovered.

Following are given the records of a number of mares which were inoculated intrauterally with saline suspensions of encapsulated bacilli in gelatine capsules. These mares were all cultured several times previous to inoculation and were known not to be suffering with this type of infection. At the end of the experiment the mares were killed and the genital organs cultured.

A. Mares Inoculated With Encapsulated Organisms Recovered From Field Cases of Metritis

No. 2577

3-25-24 Cultures from os uteri negative. 5 c. c. of suspension E1 placed in uterus.

4-824 Encapsulated bacilli on cultures from osuteri.

4-15-24 Encapsulated bacilli on cultures from os uteri.

5-9-24 Mare killed and genital tract examined. Uterus enlarged, containing a large amount of milky white viscid exudate. Cervix

was congested and open, body of uterus slightly congested at posterior part, anterior part and horns congested and hemorrhagic. The submucosa contained many small abscesses. Encapsulated bacilli were recovered in pure culture from os uteri, body and horns of uterus.

No. 4131

2-15-26 Apparently normal. Cultures from os uteri negative.
2-20-26 Apparently normal. Cultures from os uteri negative.
2-26-26 Apparently normal. Cultures from os uteri negative.
3-23-26 Apparently normal. Cultures from os uteri negative. 3 c. c. of a saline suspension of E22 placed in uterus.
3-30-26 Cervix congested, purulent exudate in uterus and vagina. Encapsulated bacilli on cultures.
4-6-26 Cervicitis exudate in uterus. Encapsulated bucilli on cultures.
4-13-26 Mucuos membranes congested, very little exudate. Encapsulated bacilli on cultures.
4-28-26 Cervix and vagina congested, exudate in uterus. Cultures covered with encapsulated bacilli.
5-3-26 Mare killed. Encapsulated bacilli recovered from cervix, body of uterus, right and left horns. Cultures from inguinal lymph glands, right and left fallopian tubes, (right and left) ovaries negative.

No. 5600

11-4-26 Apparently normal. Cultures from os uteri negative.
11-9-26 Apparently normal. Cultures from os uteri negative. 10 c. c. of a saline suspension of E9 placed in uterus.
11-11-26 Mucous membranes congested, os uteri inflamed and relaxed, large amount of muco-purulent exudate in vagina. Cultures covered with encapsulated bacilli.
11-29-26 Os uteri congested and greatly relaxed. Slight exudate in vagina. Cultures covered with encapsulated bacilli.
12-8-26 Cervix moist and congested. Cultures negative.
12-14-26 Cervix moist and congested. Encapsulated bacilli on cultures.
1-6-27 Cervix moist and congested. Cultures negative.
2-2-27 Cervix moist and congested. Not cultured.
2-4-27 Cervix moist and congested. Cultures negative.
3-28-27 Mare killed and examined. Several colonies of micrococci and streptococci on cultures from cervix and uterus. Culture from left and right horns, left and right fallopian tubes, left and right ovaries negative.

No. 5487

10-13-26 Apparently normal. Cultures from os uteri negative.
10-16-26 Apparently normal. Cultures from os uteri negative. 5 c. c. of a saline suspension of E24 placed in uterus.
10-22-26 Apparently normal. Cultures negative.
10-30-26 Apparently normal. Cultures negative.
11-4-26 Apparently normal. Few colonies of encapsulated bacilli on cultures.
11-6-26 Killed. Genital tract cultured. Streptococcus from cervix. Other cultures negative.

No. 5213

7-25-26 Apparently normal. Cultures from os uteri negative.
8-11-26 Apparently normal. Culture from os uteri negative.
8-13-26 Apparently normal. Cultures negative. 5 c. c. of a saline suspension of encapsulated bacilli recovered from a mare which had become infected in the field, placed in uterus.

Shortly after inoculation this mare showed a profuse muco-purulent exudation from the vagina with clinical evidence of cervicitis and metritis. No cultures were taken until six weeks elapsed.

9-28-26 Oestrus. Slight exudate containing flocculi in Vagina. Os uteri enlarged and congested. Cultures negative.
11-4-26 Killed and genital tract examined. Uterus thickened and edematous. Capillaries of mucosa congested. All cultures negative.

No. 3653

10-14-25 Apparently normal. Few colonies Bact. coli on cultures from os uteri.
10-17-25 Apparently normal. Cultures from os uteri negative. 2 c. c. of a saline suspension of E2 placed in uterus.
10-28-25 Slight cervicitis. Cultures negative.
10-31-25 Apparently normal. Cultures negative.
11-7-25 Apparently normal. Cultures negative. 5 c. c. of a saline suspension of E2 placed in uterus.
11-10-25 Slight cervicitis and vaginitis. Few colonies of encapsulated bacilli on cultures.
11-14-25 Apparently normal. Few colonies of non-hemolytic streptococci on cultures.
11-28-25 Apparently normal. Cultures negative. 5 c. c. of a saline suspension of E1 placed in uterus.
12-1-25 Cervicitis and vaginitis. Large amount of muco-purulent exudate. Numerous colonies of encapsulated bacilli on cultures.
12-12-25 Apparently normal. Cultures negative.
12-21-25 Oetrus. Cervicitis and vaginitis. Large amount of muco-purulent exudate. Encapsulated bacilli on cultures.
1-4-26 Apparently normal. Cultures negative.
2-10-26 Apparently normal. Cultures negative.
2-20-26 Apparently normal. Cultures negative.
2-26-26 Apparently normal. Non-hemolytic streptococci on cultures.
3-18-25 Mare killed and genital tract examined. Chronic metritis. All cultures from genital organs negative.

B. Mares Inoculated With Cultures of Granuloma Bacilli

No. 4133

2-3-26 Apparently normal. Cultures from os uteri negative. Inoculated with 5 c. c. of saline suspension of G2.
2-10-26 Apparently normal. Cultures negative.
2-16-26 Apparently normal. Cultures negative.
3-31-26 Apparently normal. Cultures negative.

Since this mare gave no clinical or bacteriological evidence of infection during the period noted above she was considered not to be

infected. She was not killed at this time but was used together with No. 4132 and No. 4131 in an experiment to demonstrate the transmission of infection with **Encapsulatus genitalium** thru breeding.

No. 4132

Mare suffering with streptococcic metritis.
2-10-26 Cervitis. Cultures negative.
2-12-26 Cultures negative. 5 c. c. of a saline suspension of G6 placed in uterus.
2-20-26 Few colonies of streptococci on cultures.
2-26-26 Cultures negative.
3-31-26 Cultures negative.

No. 4130

Mare suffering with streptococcic metritis.
2-10-26 Cervitis, metritis, os relaxed. Streptococci on cultures.
2-12-26 Streptococci on cultures. 5 c. c. of a saline suspension of G6 placed in uterus.
2-15-26 Streptococci on cultures.
2-20-26 Streptococci on cultures.
2-26-26 Streptococci on cultures.
3-18-26 Killed and genital tract examined. Streptococci recovered from cervix and body of uterus. Other organs negative. This mare was suffering with a very severe streptococcic infection. The clinical symptoms remained unchanged thruout the experiment.

Mares Inoculated With Friedlander's Bacilli

No. 4131

2-10-26 Apparently normal. Cultures from os uteri negative.
2-12-26 Apparently normal. Cultures negative. 5 c. c. of a saline suspension of F1 placed in the uterus.
2-15-26 Apparently normal. Cultures negative.
2-20-26 Apparently normal. Cultures negative.
2-26-26 Apparently normal. Cultures negative.
3-23-26 Apparently normal. Cultures negative.

No. 4139

Mare suffering with streptococcic metritis.
2-10-26 Streptococci on cultures from os uteri.
2-12-26 Streptococci on cultures. 5 c. c. of a saline suspension of F1 placed in uterus.
2-15-26 Streptococci on cultures.
2-20-26 Streptococci on cultures.
2,26-26 Streptococci on cultures.
3-18-26 Mare killed and reproductive organs examined. Streptococci from cervix, body of uterus, left and right horns and right fallopian tube. Left fallopian tube and ovaries yielded no growth.

This mare was suffering with a severe streptococcic metritis when received. The vagina and cervix were slightly inflamed, the cervix was relaxed and there was a purulent exudate in the uterus. Encapsulated bacilli were not recovered on cultures after inoculation

of Friedlander's bacillus into the uterus. The clinical symptoms remained unchanged thruout the course of the experiment.

No. 5214

7-25-26 Apparently normal. Cultures from os uteri negative.
8-11-26 Apparently normal. Cultures negative.
9-14-26 Apparently normal. Cultures negative.
9-15-26 10 c. c. of a saline suspension of F H M* placed in uterus.
9-18-26 Apparently normal. Cultures negative.
9-20-26 Bloody exudate in cervix. Cervix slightly relaxed and inflamed. Cultures negative.
9-22-26 Estruation. Cervix slightly relaxed, serous exudate. Cultures negative.
10-1-26 Adhesions formed in cervix broken down. Cultures negative.
10-5-26 Cervix inflamed. Cultures negative.
2-7-27 Killed and reproductive organs examined. Genital tract apparently normal. All cultures negative.

This mare had a very small, tightly contracted cervix. It was irritated in placing the capsule in the uterus, probably causing the formation of adhesions.

*FHM was a Friedlander's bacillus from a case of human pneumonia. It had been under artificial culture only two months when used in this experiment.

No. 5704

Mare suffering with streptococcic metritis.
10-4-26 Vaginitis and cerviciti s. Cultures from os uteri negative.
10-7-26 Cultures negative. 10 c. c. of a saline suspension of F6 placed in uterus.
10-11-26 Vaginitis and cervicitis. Encapsulated bacilli on cultures.
10-13-26 Vaginitis and cervicitis. Approximately 1000 c. c. of a very viscid muco-purulent exudate in the vagina. Encapsulated bacilli on cultures.
10-22-26 Mare discharging muco-purulent exudate from vagina. Cervicitis. Encapsulated bacilli on cultures.
10-30-26 Cervix congested, moist. Cultures negative.
11-4-26 Estruation. Cervicitis, vaginitis. Cervix moist. Encapsulated bacilli on cultures.
11-11-26 Vagina and cervix red and congested. Cervix tightly contracted. Encapsulated bacilli on cultures.
11-30-26 Mare killed and reproductive organs examined. Vagina and uterus contained a large amount of exceedingly tenacious, muco-purulent exudate. Mucous membrane of the uterus was uniformly congested and inflamed, deep red in color. Cultures from cervix, body of the uterus and left and right horns were covered with encapsulated bacilli. Cultures from the fallopian tubes and ovaries were negative.

This mare was known to be suffering with streptococcic metritis when received at the laboratory. At this time the cervix was inflamed and the mucous membrane congested. After inoculation with Friedlander's bacillus (F6) the inflammation rapidly increased and a profuse exudation was set up.

No. 6359

Mare suffering with streptococcic metritis.

10-4-26 Cervicitis, slight mucous exudate. Cultures from os uteri negative.

10-7-26 Cervicitis, slight mucous exudate. Streptococci on cultures. 10 c. c. of a saline suspension of F26 placed in uterus.

10-11-26 Vaginitis and cervicitis. Large amount of muco-purulent exudate in vagina. Encapsulated bacilli on cultures.

10-13-26 Vaginitis and cervicitis. Muco-purulent exudate in vagina. Encapsulated bacilli on cultures.

10-22-26 Vaginitis and cervicitis. Slight exudate from cervix. Encapsulated bacilli on cultures.

10-22-26 Vaginitis and cervicitis. Slight exudate from cervix. Encapsulated bacilli on cultures.

10-30-26 Cervix slightly congested, moist. Encapsulated bacilli on cultures.

11-4-26 Cervicitis and vaginitis, slight exudate from cervix. Cultures negative.

11-11-26 Cervicitis. Very marked congestion of mucous membrane of vagina. Encapsulated bacilli on cultures.

11-17-26 Mare killed and genital organs examined. Uterus congested and inflamed. Approximately 50 c. c. of a muco-purulent exudate in vagina. Cultures from cervix, body of uterus, left and right horns yielded numerous colonies of encapsulated bacilli. Fallopian tubes and ovaries not cultured.

This mare, like No. 5704, exhibited cervicitis before inoculation with Friedlander's bacillus. However, the symptoms were greatly aggravated and exudation became profuse after the inoculation with encapsulated organisms.

No. 5599

11-4-26 Apparently normal. Cultures negative. 10 c. c. of a saline suspension of F26 placed in uterus.

11-11-26 Vaginitis and cervicitis. Cultures covered with encapsulated bacilli.

11-19-26 Apparently normal. One colony of encapsulated bacilli on cultures.

11-29-26 Apparently normal. Few colonies of encapsulated bacilli on cultures.

12-8-26 Cervicitis, slight exudate. Encapsulated bacilli on cultures.

1-6-27 Cervicitis, slight exudate. Few colonies of encapsulated bacilli on cultures.

2-4-27 Cervicitis, vaginitis. Exudate in Vagina. Encapsulated bacilli on cultures.

2-8-27 Killed and reproductive organs examined. Cervix and uterus apparently normal. Encapsulated bacilli on cultures from cervix; other organs negative.

No. 2798

10-22-26 Apparently normal. Cultures from os uteri negative.

10-26-26 Estruation. Cervix relaxed. Slight serious exudate.

Cultures negative. 5 c. c. of a saline suspension of F25 placed in uterus.

10-30-26 Apparently normal. Cultures negative.

11-4-26 Apparently normal. Cultures negative.

11-11-26 Slight cervicitis and vaginitis. Cultures negative.

11-19-26 Vaginitis and cervicitis. Muco-purulent exūdate in vagina. Encapsulated bacilli on cultures.

11-29-26 Vaginitis and cervicitis. Cervix relaxed. Slight exudate from cervix. Encapsulated bacilli on cultures.

12-8-26 Vaginitis and cervicitis. Small amount of exudate in vagina. Few colonies of encapsulated bacilli on cultures.

1-6-27 Cervicitis. Slight exudate from cervix. Encapsulated bacilli on cultures.

2-2-27 Vaginitis, cervicitis. Slight exudate from cervix. Encapsulated bacilli on cultures.

2-23-27 Mare killed and reproductive organs examined. Cervix congested and moist. Large amount of muco-purulent exudate in uterus. Encapsulated bacilli recovered from cervix, body of uterus, left and right horns, right fallopian tube, left and right ovaries. Cultures from left fallopian tube negative. The organisms recovered were found to belong to the same serological type as F25, the culture introduced. This is not the type found in field cases of metritis.

D. Mares Inoculated With Bact. Aerogenes

No. 5215

7-25-26 Apparently normal. Cultures from os uteri negative.

8-11-26 Apparently normal. Cultures negative.

9-22-26 Apparently normal. Cultures negative.

9-25-26 Apparently normal. Cultures negative. 5 c. c. of a saline suspension of A3* place in uterus.

9-28-26 Estruation. Cervicitis, large amount of muco-purulent exudate in vagina. Encapsulated bacilli on cultures.

10-1-26 Estruation, cervix inflamed, slight serous exudate. Cultures negative.

10-5-26 Cervix slightly congested. Cultures negative.

10-11-26 Apparently normal. Cultures negative.

10-22-26 Estruation. Cervix slightly congested and relaxed. Slight serious exudate. Cultures negative.

10-30-26 Apparently normal. Cultures negative.

11-4-26 Cervix slightly congested. Encapsulated bacilli on cultures.

11-11-26 Estruation. Cervix reduced and relaxed, serous exudate. Cultures negative.

11-19-26 Apparently normal. Cultures negative.

11-29-26 Apparently normal. Cultures negative.

12-2-26 Mare killed and reproductive organs examined. Uterus thickened and edematous. All cultures negative.

No. 1657

Mare suffering with streptococcic metritis.

4-7-27 Cervicitis. Streptococci on cultures from cervix.

5-18-27 Cervix slightly congested. Cultures negative.

5-20-27 Cervix slightly congested. Cultures negative. 10 c. c. of a saline suspension of A3* placed in uterus.

5-25-27 Vaginitis and cervicitis. Small amount of exudate in vagina, Encapsulated bacilli on cultures.

6-6-27 Vaginitis and cervicitis. Purulent exudate from cervix. Encapsulated bacilli on cultures.

6-21-27 Estruation. Cervix congested, moist. Small amount of purulent exudate in vagina. Cultures covered with encapsulated bacilli.

7-8-27 Mare killed and reproductive organs examined. Encapsulated bacilli recovered from cervix, uterus and right ovary. Cultures from fallopian tubes and left ovary were negative. Cervix moist and congested. Small amount of muco-purulent exudate in uterus.

Included in the cases given above are eighteen mares which were inoculated with encapsulated bacilli from various sources. Six of these mares received cultures of *Encapsulatus genitalium*, seven were inoculated with Friedlander's bacillus, organisms recovered from cases of inguinal granuloma were placed in the uteri of three, and two were inoculated with *Bact. aerogenes*. In each case the clinical condition of the mare before and after inoculation and the results of the bacteriological examinations are given. The post-mortem findings are included in all but three cases. From the cases cited above it is evident that under the condition of these experiments it is possible to artificially infect mares with pure cultures of encapsulated bacilli. Not only are the organisms recovered from cases of metritis in mares capable of reproducing this condition, but Friedlander's bacillus and *Bact. aerogenes* may cause an inflammation of the genital tract when placed in the uterus.

It is evident from the infection experiments that mares suffering with streptococcic metritis are much more susceptible to infection with encapsulated bacilli than are mares which are apparently normal. In the mares suffering with streptococcic metritis the clinical changes following the inoculation with encapsulated bacilli are much more pronounced and the organisms persist for a much longer time.

In these experiments we have found that the ability of encapsulated organisms to persist and initiate pathologic changes

* A3 is a soil strain of Bact. aerogenes. It produces a heavy capsule and belongs to the same agglutinative type as the cultures of encapsulated bacilli isolated from mares in the field.

in the genital tract is apparently dependent upon the production by the bacillus of a large amount of capsular substance. Only those strains producing heavy capsules and exhibiting a very mucoid, tenacious growth on artificial media have been found to be infectious. An example of this is the inoculation of mare No. 3653 with culture E2. This organism was isolated from a case of metritis in the field. After growth on artificial medium for some time it lost the property of forming large capsules and its mucoid character of growth. It was, at that time, incapable of persisting in the genital tract. The ability of these organisms to persist in the genital tract coincides very closely with their virulence for the laboratory animals. These observations confirm the results of Toennissen (10) and Julianelle (2, 3, 4) on the laboratory animals. These workers noted that virulence was dependent upon capsule formation.

The fact that none of the granuloma bacilli employed in these studies were able to persist in the genital tract is probably due to lack of capsule formation. None of the strains in our collection were vigorous capsule formers, all being old laboratory strains.

Of the six mares inoculated with cultures of *Encapsulatus genitalium* only two yielded positive cultures on post-mortem examination. In general the metritis, produced in these cases was much less severe and less persistent than that found in naturally infected cases occurring in the field. This difference, we believe, is explained by the relative susceptibility of the two classes of mares. The mares becoming infected in the field are, in the large majority of cases, mares which have been previously suffering with metritis due to streptococci or are foaling mares. Both foaling mares and mares suffering with streptococcic metritis are extremely susceptible to this form of infection. The genital tract in these conditions offers the encapsulated bacilli a most favorable medium for growth. Non-infected barren mares are much more resistant. The genital tract is normal and presents an unbroken, healthy mucous membrane which is, in our experience, relatively resistant to invasion by microorganisms.

Summary. It is impossible to differentiate the equine cultures from certain cultures of the Friedlander bacilli by the tests used in these studies. The equine cultures all belong to a single homogeneous type. The Friedlander bacilli, as represented by the cultures in our possession, are an ill-defined heterologous group. However, certain of the Friedlander strains are morphologically, culturally, biochemically and serologically identical with the organisms recovered from metritis in mares. In addition, one strain of *Bact. aerogenes* (A3) has been found to belong to this type. Other strains of *Bact. aerogenes* have been found which possessed all the attributes generally attributed to Friedlander's bacillus, which we have found it impossible to differentiate from strains recovered from infections in humans and which have been called Friedlander's bacilli. Further, it is impossible to differentiate these bacilli by their action on laboratory animals or by testing their ability to produce metritis when introduced into the genital tract of mares. Certain strains of *Bact. aerogenes, Encapsulatus pneumoniae,* and *Encapsulatus inguinalis* are not separable by the tests employed here. Such a condition demonstrates the need for further studies in the genus Encapsulatus and the genus Aerobacter, of which *Bact. aerogenes* is the type species. The separation of these organisms into two genera does not, in the light of our present knowledge, seem justifiable.

The Transmission of Encapsulatus Genitalium Infection Thru Breeding

In the majority of the cases of infection with *Encapsulatus genitalium* occurring in the field, the source of infection may be traced to breeding. In order to establish the transmissibility of this infection the following experiment was performed:

Three mares known not to be carriers of encapsulated bacilli were used. A pure culture of an encapsulated organism (E22) was placed in the uterus of one of these mares. After the infection had become established in this mare (7 days) she was bred to stallion No. 14. Later, on the same day, the two non-infected

mares were bred to the stallion. Three days later this procedure was repeated. The mares were then examined several times and post-mortem examinations held.

In Table 8 are given the results of this experiment:

TABLE NO. 8

Date	No. 4131	No. 4133	No. 4132
2-15-26	Apparently normal. Cultures negative.	Apparently normal. Cultures negative.	Cervicitis. Cultures negative.
2-20-26	Apparently normal. Cultures negative.	Apparently normal. Cultures negative.	Cervicitis. Streptococci on cultures.
2-26-26	Apparently normal. Cultures negative.	Apparently normal. Cultures negative.	Cervicitis. Cultures negative.
3-23-26	3 c. c. of saline suspensision E 22 placed in uterus.		
3-30-26	Estruation Cervicitis. Vaginitis. Profuse muco-purulent exudate. Encapsulated bacilli in cultures.		
3-31-26	Bred to stallion No. 14.	Estruation. Apparently normal. Cultures negative. Bred to stallion No. 14 after he had served mare No. 4131.	Estruation. Cervicitis. Cultures negative. Bred to stallion No. 14 after he had served mares No. 4131 and No. 4133.
4-2-26	Bred to stallion No. 14.	Bred to stallion No. 14 after he had served mare No. 4131.	Bred to stallion No. 14 after he had served mares No. 4131 and No. 4133.
4-6-26	Cervicitis, vaginitis, exudate. Encapsulated bacilli on cultures.	Cervicitis, vaginitis, slight exudate. Encapsulated bacilli on cultures.	Cervicitis, vaginitis, exudate in vagina. Encapsulated bacilli on cultures.
4-8-26	Pure cultures of encapsulated bacilli recovered from gum on penis of stallion (methyl violet agar.)		
4-13-26	Cervicitis, mucous membranes congested. Slight exudate. Encapsulated bacilli on cultures.	Cervicitis, mucous membranes congested. Slight exudate. Encapsulated bacilli on cultures.	Cervicitis, cervix relaxed, mucous membranes congested. Large amount of muco-purulent exudate in vagina. Encapsulated bacilli on cultures.
4-28-26	Cultures from gum on penis of stallion on methyl violet agar negative. Few colonies of streptococcus on cultures from urethra.		

TABLE NO. 8—Continued.

Date	No. 4131	No. 4133	No. 4132
4-28-26	Cervicitis, mucous membranes congested. Slight exudate from cervix. Encapsulated bacilli on culture.	Cervicitis, mucous membranes congested. Exudate in vagina. Encapsulated bacilli on cultures.	Cervicitis, mucous membranes congested. Exudate in vagina. Encapsulated bacilli on cultures.
5-23-26	Killed— Vaginitis, chronic cervicitis, metritis, hemorrhagic areas in epithelium of uterus. Fallopian tubes thickened, evidence of salpingitis. Encapsulated bacilli recovered from cervix, body of uterus, left and right horns. Cultures from fallopian tubes and ovaries negative.	Killed— Cervicitis, cervix relaxed and atonic. Metritis, congested areas in epithelium of uterus. Fallopian tubes apparently normal. Ovaries small and indurated. Encapsulated bacilli recovered from cervix, body of uterus, left and right horns, left and right fallopian tubes, left and right ovaries.	Killed— Cervicitis, cervix congested and inflamed. Metritis, mucosa deep purplish color. 250 c. c. mucopurulent exudate in uterus. Salpingitis, fallopian tubes thickened. Inguinal lymph glands degenerated, filled with white mucoid, gummy exudate. Encapsulated bacilli recovered from cervix, body of uterus, right and left horns, right and left fallopian tubes, per-ovarian cyst, left ovary and inguinal lymph glands.

Field Cases of Infection Thru Breeding

1. Mare No. 2333. In October, 1924, the mare was examined for pregnancy and found barren. Culture tubes inoculated from cervix and uterus yielded *Encapsulatus genitalium* and *Ps. Pyocyanea.* On May 5, 1925, was bred to stallion No. 63 Mare No. 3848, with foal at side, was bred to stallion No. 63 on May 6, 1925. Mare No. 3849, with foal at side, was bred to stallion No. 63 on May 7, 1925. Mares No. 3848 and 3849 were examined for pregnancy Nov. 20, 1925. Both were barren and clinical examination revealed that they were suffering with metritis. Culture tubes inoculated from cervix and uterus yielded *Encapsulatus genitalium* in both cases.

This field case closely approximates the breeding experiment carried out at the laboratory. Two foaling mares bred on consecutive days to a stallion who, on the previous day, had covered a mare infected with *Encapsulatus genitalium,* both contracted the infection.

2. Mare No. 2359 was known to have been infected with *Encapsulatus genitalium* since February, 1924. She was bred to stallion No. 72 early in the spring of 1925. The mare failed to conceive and was bred to the same stallion a number of times during the season. Mares No. 2390, No. 2444, No. 2445, and No. 2927 were examined in the fall of 1924 and were not infected with *Encapsulatus genitalium* at that time. These mares were also bred to stallion No. 72 during the spring of 1925. Since they failed to conceive they were bred repeatedly. When examined in the fall of 1925 all were found to be infected with *Encapsulatus genitalium.*

Mares No. 5241, No. 5242, No. 5243 and No. 5424 were examined for the first time August 3, 1926. All were infected with *Encapsulatus genitalium.* This latter group of mares had been bred to or impregnated from stallion No. 72 during the breeding season of 1925 and 1926.

The cases cited above are typical of the transmision of this infection in the field and demonstrate the extreme ease with which the disease may be contracted thru breeding.

Inoculation of Stallions With Encapsulatus Genitalium

In studying the transmission of *Encapsulatus genitalium* in the field it is necessary to consider the role played by the stallion in the spread of the disease. Other workers have studied the spread of genital disease by the stallion.

Dimock and Snyder (12) reported the presence of streptococci in the semen of 88% of 36 stallions examined. Williams (13) cites the case of a stallion that harbored infection in the genital tract. This horse was able to produce pregnancy in only a low percentage of the mares which he served. After being bred to this horse many of the mares failed to conceive when bred to normal stallions.

In the present studies efforts have been made to artificially infect stallions with *Encapsulatus genitalium* thru the injection of pure cultures of the organism into the urethra. After the injection of the bacilli, culture tubes were inoculated from the urethra in an effort to recover the organism.

Following are given the results of intraaurethral injection of *Encapsulatus genitalium* in two stallions:

Stallion No. 6445

10-20-26 Culture tubes inoculated from urethra, negative.
11-2-26 Culture tubes inoculated from urethra, negative.
11-4-26 10 c. c. of a saline suspension of E9 injected into urethra.
11-12-26 Culture tubes inoculated from urethra, negative.
12-2-26 Killed and genital tract examined, apparently normal. Culture tubes inoculated from urethra, seminal glands and testes negative.

Stallion No. 4443

9-16-26 Culture tubes inoculated from urethra, negative.
9-18-26 10 c. c. of a saline suspension of E1 injected into urethra.
9-28-26 Culture tubes innoculated from urethra, negative.
10-1-26 10 c. c. of saline suspension of E9 injected into urethra.
10-5-26 Culture tubes inoculated from urethra, negative.
10-9-26 Killed and genital organs examined. Apparently normal. Culture tubes inoculated from urethra, seminal glands, and testes negative.

The experiments cited above indicate that stallions do not easily become infected. These results are in accord with observations made in the field. It is our opinion that the stallion

does not become infected with encapsulated bacilli, but acts merely as a passive carrier, the organisms persisting on the penis for only a short time.

REPORTS OF TYPICAL CASES

In the following case reports we have given the histories of thirteen barren mares. These are animals that have been examined many times over a number of years and are typical of the classes of cases which they represent. Three of the mares represent that class of non-breeding animals which are not suffering from genital infection but have physiological abnormalities of the reproductive organs. Five of the mares were suffering with streptococcic infections, four were infected with *Encapsulatus genitalium,* and with one the results of the bacteriological studies were inconclusive.

Barren Mares Consistently Negative on Bacteriological Examination

No. 1226

1922

4-24-22 Culture tubes (3) inoculated from cervix and uterus, negative.

5-1-22 Culture tubes (2) inoculated from cervix and uterus, negative. Bred spring 1922.

10-10-22 (Barren) Culture tubes inoculated from cervix and uterus, negative.

10-31-22 Culture tubes inoculated from cervix and uterus, negative. Bred Spring 1923, failed to conceive. Killed February 20, 1924. Reproductive organs examined. Culture tubes inoculated from cervix, uterus, apices of horns, fallopian tubes and ovaries were negative. Ovaries enlarged, chronic fibrous, nineteen small multiple cysts in one ovary. In neither ovary could any normal ovarian tissue be detected. This case was diagnosed on clinic examination as ovarian trouble. Estrual periods were irregular in occurrence and duration.

No. 2995

1924

Normal foal spring 1924. Bred spring 1924, failed to conceive.

10-24-24 Culture tubes inoculated from cervix and uterus, negative.

10-28-24 Culture tubes inoculated from cervix and uterus, negative.

1925

1-12-25 Culture tubes inoculated from cervix and uterus, negative. Bred spring 1925, failed to conceive.

1926

Bred spring 1926, failed to conceive.

10-23-26 Culture tubes inoculated from cervix and uterus, negative.

1927

1-4-27 Culture tubes inoculated from cervix and uterus, negative.

2-24-27 Culture tubes inoculated from cervix and uterus, negative. Bred.

May 1927, Examined for pregnancy—in foal. Cervical membrane swollen, congested, edmatous and moist. Ovaries enlarged, cystic, estrual period irregular in frequency and duration. Treatment—only treated during winter 1927. Hypodermic injections of glandular extracts.

No. 2277

1923

Bred spring 1923, failed to conceive.

12-7-23 Condition of genital tract suggested possibility of infection. Culture tubes inoculated from cervix and uterus, negative.

10-28-23 Culture tubes inoculated from cervix and uterus, negative.

1924

1-4-24 Culture tubes inoculated from cervix and uterus, negative.
1-25-24 Culture tubes inoculated from cervix and uterus, negative.
1-30-24 Culture tubes inoculated from cervix and uterus, negative.
1-30-24 Culture tubes inoculated from cervix and uterus, negative.
2-15-24 Culture tubes inoculated from cervix and uterus, negative.
2-22-24 Culture tubes inoculated from cervix and uterus, negative.
3-4-24 Culture tubes inoculated from cervix and uterus, negative.
3-18-24 Culture tubes inoculated from cervix and uterus, negative.

10-27-24 Culture tubes inoculated from cervix and uterus, negative.

12-1-24 Culture tubes inoculated from cervix and uterus, negative.

1925

Bred spring 1925. Aborted February 1926.

1926

2-7-26 Culture tubes inoculated from cervix and uterus, negative.

2-16-26 Culture tubes inoculated from cervix and uterus, negative. Bred spring 1926, conceived. Aborted twins March 29th, 1927. Both fetuses cultured negative.

1927

3-14-27 Culture tubes inoculated from cervix and uterus, negative.
3-25-27 Culture tubes inoculated from cervix and uterus, negative.
5-3-27 Culture tubes inoculated from cervix and uterus, negative.

Treatment—Uterine douches during fall, 1926 and winter of 1927. Ovaries punctured in May, 1927, and several cunces of serous fluid removed.

Barren Mares Showing Infection of Genital Tract Over Long Period of Time—Streptococcus

No. 1800

1923

2-16-23 Culture tubes inoculated from cervix and uterus, treptococcus. Mare bred six times during spring 1923 and failed to conceive.

1924

1-29-24 Culture tubes inoculated from cervix and uterus, streptococcus.

3-31-24 Culture tubes inoculated from cervix and uterus, miscellaneous.

5-1-24 Culture tubes inoculated from cervix and uterus, streptococcus.

5-22-24 Culture tubes inoculated from cervix and uterus, streptococcus. Bred spring, 1924, and failed to conceive.

9-12-24 Culture tubes inoculated from cervix and uterus, negative.

9-19-24 Culture tubes inoculated from cervix and uterus, negative.

9-29-34 Culture tubes inoculated from cervix and uterus, streptococcus.

1925

1-29-25 Culture tubes inoculated from cervix and uterus, streptococcus. Bred spring of 1925 and conceived. Normal foal in spring of 1926.

6-28-26 Bred and conceived.

1927

1-31-27 Aborted. Streptococcus isolated from fetus.

3-17-27 Culture tubes incculated from cervix and uterus, miscellaneous.

No. 3159

Examined for pregnancy 11/20/23—positive. Aborted fall, 1923, or winter, 1924. No record of an examination of the mare during 1924. Bred spring, 1924, conceived. Aborted January 9th, 1925. Examined January 10th, 1925. Streptococci isolated from uterine exudate.

Jan. 22-25 Culture tubes inoculated from the cervix and uterus, negative. Bred spring, 1925, conceived. Foaled prematurely Feb. 7, 1926 and foal died Feb. 12, 1926.

Mch. 11-26 Culture tubes inoculated from cervix and uterus, streptococci.

Mch. 16-26 Culture tubes inoculated from cervix and uterus, streptococci. Bred spring 1926, conceived. Gave birth to live foal spring 1927. Afterbirth showed marked evidence of disease (necrosis) on one horn of chorion. Foal was small in size and defective in conformation; however, is doing nicely and will possibly develop into a useful animal.

No. 2388

1924

1-29-24 Culture tubes inoculated from cervix and uterus, negative.

2-1-24 Culture tubes inoculated from cervix and uterus, streptococcus.

3-31-24 Culture tubes inoculated from cervix and uterus, streptococcus.
5-1-24 Culture tubes inoculated from cervix and uterus, streptococcus.
5-26-24 Culture tubes inoculated from cervix and uterus, streptococcus. Bred spring 1924, failed to conceive.
9-12-24 Culture tubes inoculated from cervix and uterus, negative.
9-19-24 Culture tubes inoculated from cervix and uterus, negative.
10-20-24 Culture tubes inoculated from cervix and uterus, negative.
10-30-24 Culture tubes inoculated from cervix and uterus, streptococcus.
11-12-24 Culture tubes inoculated from cervix and uterus, negative.

1925.
1-29-25 Culture tubes inoculated from cervix and uterus, negative. Bred spring 1925, failed to conceive.
11-27-25 Culture tubes inoculated from cervix and uterus, miscellaneous.
2-13-26 Culture tubes inoculated from cervix and uterus, streptococcus.
5-8-26 Culture tubes inoculated from cervix and uterus, negative Bred spring, 1926, failed to conceive.
10-2-26 Culture tubes inoculated from cervix and uterus, negative. Has been under treatment from January, 1924, to June, 1927.

No. 956

1921
10-31-21 Culture tubes inoculated from cervix and uterus, negative.
11-14-21 Culture tubes inoculated from cervix and uterus, miscellaneous.

1922
4-20-22 Culture tubes inoculated from cervix and uterus, streptococcus.
5-10-22 Culture tubes inoculated from cervix and uterus, streptococcus.
6-10-22 Culture tubes inoculated from cervix and uterus, streptococcus.
9-21-22 Culture tubes inoculated from cervix and uterus, short rod.
11-22-22 Culture tubes inoculated from cervix and uterus, short rod.

1923
1-8-23 Culture tubes inoculated from cervix and uterus, short rod.
1-26-23 Culture tubes inoculated from cervix and uterus, short rod.
2-5-23 Culture tubes inoculated from cervix and uterus, negative.
2-19-23 Culture tubes inoculated from cervix and uterus, negative.
3-7-23 Culture tubes inoculated from cervix and uterus, negative.
3-15-23 Culture tubes inoculated from servix and uterus, negative. Bred April, 1923, conceived. Had normal foal March 17, 1924.

No. 1581

1922

11-25-22 Culture tubes inoculated from cervix and uterus, miscellaneous.

1923

2-28-23 Culture tubes inoculated from cervix and uterus, miscellaneous.

3-9-23 Culture tubes inoculated from cervix and uterus, streptococcus.

4-24-23 Culture tubes inoculated from cervix and uterus, miscellaneous. Bred spring, 1923, failed to conceive.

12-7-23 Culture tubes inoculated from cervix and uterus, streptococcus.

1924

1-30-24 Culture tubes inoculated from cervix and uterus, negative.

3-7-24 Culture tubes inoculated from cervix and uterus, streptococcus. Bred spring, 1924, failed to conceive.

10-27-24 Culture tubes inoculated from cervix and uterus, negative.

1925

Bred in spring, 1925. Conceived and gave birth to normal foal 1926.

1926

Bred in spring, 1926. Conceived and gave birth to normal foal, 1927. Died from ruptured stomach, April, 1927. Treatment—Under treatment and observation from November 20th, 1923, until she was bred and conceived in spring, 1925.

Barren Mares Showing Infection of Genital Tract Over Long Period of Time—*Encapsulatus genitalium*

No. 2134

1923

10-19-23 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.

11-1-23 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.

11-8-23 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.

12-6-23 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.

12-17-23 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.

12-27-23 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.

12-31-23 Culture tubes inoculated from cervix and uterus, contamination.

1924

1-24-24 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.

1-28-24 Culture tubes inoculated from cervix and uterus, mold.
2-18-24 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.
3-24-24 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.
3-31-24 Culture tubes inoculated from cervix and uterus, negative.
4-9-24 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.
4-21-24 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.
4-28-24 Culture tubes incculated from cervix and uterus, Encapsulatus genitalium.
5-2-24 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.

No. 2359

Aborted twin fetuses in fall of 1921, normal foal in 1923, and aborted twins January 10th, 1924.

1924

1-21-24 Cultures from exudate in vaginal canal, streptococcus. Cultures from cervix and uterus, negative.
1-28-24 Culture tubes inoculated from cervix and uterus, negative.
2-18-24 Culture tubes inoculated from cervix and uterus, negative.
2-25-24 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.
2-28-24 Culture tubes inoculated from cervix and uterus, negative.
3-7-24 Culture tubes inoculated from cervix and uterus, negative.
3-13-24 Culture tubes inoculated from cervix and uterus, negative.
3-24-24 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.
3-31-24 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.
4-21-24 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.
4-28-24 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.
5-2-24 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.
11-17-24 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.

1925

Bred spring 1925 and failed to conceive.

1926

1-15-26 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.
5-19-26 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium. Bred spring of 1926 and failed to conceive.
10-2-26 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.
12-22-26 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.

1927

1-18-27 Culture tubes inoculated from cervix and uterus, negative.
2-10-27 Culture tubes inoculated from cervix and uterus, Staph. albus.
4-10-27 Culture tubes inoculated from cervix and uterus, Staph. albus.
5-2-27 Culture tubes inoculated from cervix and uterus, Staph. albus.

No. 1459

1922

9-26-22 Culture tubes inoculated from cervix and uterus, Ps. pyocyanea.
10-3-22 Culture tubes inoculated from cervix and uterus, Ps. pyocyanea.
10-25-22 Culture tubes inoculated from cervix and uterus, Ps. pyocyanea.
10-31-33 Culture tubes inoculated from cervix and uterus, Ps. pyocyanea.
11-16-22 Culture tubes inoculated from cervix and uterus, Ps. pyocyanea.

1923

1-18-23 Culture tubes inoculated from cervix and uterus, negative.
3-10-23 Culture tubes inoculated from cervix and uterus, Ps. pyocyanea.
3-26-23 Culture tubes inoculated from cervix and uterus, Ps. pyocyanea.
4-23-23 Culture tubes inoculated from cervix and uterus, negative.
5-1-23 Culture tubes inoculated from cervix and uterus, streptococcus.
5-24-23 Bred and failed to conceive.
10-19-23 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.
11-1-23 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.
12-17-23 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.

1924

1-3-24 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.
1-14-24 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.
1-18-24 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.
2-28-24 Culture tubes inoculated from cervix and uterus, negative.
3-14-24 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.
3-31-24 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.
11-17-24 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.

1925

5-11-25 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium. Bred in spring of 1925 and failed to conceive.

1926

5-8-26 Culture tubes inoculated from cervix and uterus, Streptococcus.

10-2-26 Culture tubes inoculated from cervix and uterus, Streptococcus.

1927

4-15-27 Culture tubes inoculated from cervix and uterus, Streptococcus. Mare has been under treatment constantly from September, 1922 to June, 1927.

No. 2928

1924

9-25-24 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.

11-25-24 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.

1925

2-3-25 Culture tubes inoculated from cervix and uterus, negative.

2-6-25 Culture tubes inoculated from cervix and uterus, negative.

2-13-25 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.

2-24-25 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.

3-7-25 Culture tubes inoculated from cervix and uterus, Encapsulatus genitalium.

10-31-25 Culture tubes inoculated from cervix and uterus, Streptococcus.

11-22-25 Culture tubes inoculated from cervix and uterus, negative.

1926

3-5-26 Culture tubes inoculated from cervix and uterus, negative. Bred spring of 1926 and conceived.

No. 1779

First examination March 16th, 1923. Genital tract apparently normal and culture tubes inoculated from cervix and uterus negative. Bred spring 1923. Conceived, gave birth to premature foal, Lab. No. 2623, April 14th, 1924. Streptococcus isolated from lungs.

Examination April 18th, 1924. Purulent exudate in uterus, acute cervicitis and metritis. Culture tubes inoculated from cervix and uterus showed growth of streptococci.

Bred Spring 1924. Conceived, gave birth to dead foal March 4, 1925. Foal not examined.

Examined March 7th, 1925. Exudate in uterus. Culture tubes inoculated from cervix and uterus showed extensive growth but badly overgrown with molds, making it impossible to determine the kinds of bacteria present.

Bred spring 1925. Failed to conceive. March 2nd, 1926 culture tubes inoculated from cervix and uterus badly overgrown with molds and spore formers. March 25th, 1926. Culture tubes inoculated from cervix and uterus, Streptococci.

March 7th, 1927. Culture tubes inoculated from cervix and uterus. Miscellaneous.

March 17th, 1927. Culture tubes inoculated from cervix and uterus, Streptococcus.

TREATMENT

In preparing the mare for treatment of the genital tract the same methods of confinement are used as recommended for initial examination of barren mares; also the same methods of washing and disinfecting the external parts (page 158). Where a number of infected mares on the same farm are under treatment, especially if there are mares suffering from different types of infection, the most precautionary and exacting measures must be observed.

While the diagnosis of barrenness in mares demands the most careful and exacting examination, the treatment likewise demands careful and exacting application. For barren mares, with apparently normal genital organs and with a reasonably good breeding record, no particular treatment is recommended. However, in many noninfected mares with unsatisfactory breeding records, genital abnormalities and functional disturbances may, in many cases, be corrected by the proper procedure. With infected mares it is comparatively easy to introduce medicinal agents into the uterine cavity, but the response to treatment is always slow. The selection of the medicinal agents, the method and frequency of application, and the duration of the treatment depend upon the character of the infection and the condition of the genital tract. It is very difficult to determine just when treatment may be discontinued. Ordinarily, with recently infected mares, treatment will cover a period of six months. With chronic infected cases, treatment for a period of two years may be necessary. It must be understood that the response of each case to treatment varies with the individual. When, in the opinion of a clinician who is familiar with the normal genital tract, the condition approaches normal and the bacteriological

examinations are negative, treatment should be discontinued, but the mare should be kept under observation and bacteriological examinations made from time to time. It is never advisable to breed a mare within two months after treatment is discontinued. In the great majority of cases which have been under treatment during the fall and winter, the results will be better if the mare is held over until the breeding season of the following year. Our experience has demonstrated that a definite prognosis regarding the duration and result of treatment cannot be made. The advisability of treatment depends upon the value, the age and condition of the mare, the number of mares requiring treatment, and the facilities for the proper treatment.

While the use of antiseptic solutions is an established method in the treatment of infections of the uterus, cervix, and vagina, yet their routine application without discrimination will not always produce the desired effect and may result in the loss of valuable time. The following antiseptic agents have been used by us experimentally in the treatment of genital infections of mares: Chlorinated antiseptics, proflavine and acriflavine, mercurochrome, (220 soluble) argyrol, permanganate of potash, boric acid, normal salt solution, and iodine in both aqueous and oleous solutions. Uterine capsules containing antiseptics in powdered form seemed to be beneficial, either used alone or following douching. Ichthyol, iodoform, alum, water-soluble iodine, therapogen, and other preparations have been tried, but in our experience were not satisfactory. The chlorinated antiseptics and the flavines seemed to be best for douching.

In a certain number of cases frequent and long continued douching will result in irritation, and in such cases it was our practice to temporarily discontinue douching and to use uterine capsules or iodine and oil once or twice a week, according to developments.

We were unable to observe that any one of the agents used was appreciably more effective for one type of infection than for another. Therefore an agent effective against streptococcic metritis was proportionately effective in cases of metritis due to encapsulated bacilli and other forms of infection.

SUMMARY

1. The method of making a bacteriological examination of the cervix and uterus of barren mares as outlined in this bulletin is, if properly carried out, a most reliable method of determining the presence or absence of infection in the uterus of mares.

2. The bacteriological examination of the cervix and uterus of mares is essential to determine the presence or absence of infection and to differentiate between the differnt kinds of infection.

3. Approximately one-third of all barren mares examined were found to be suffering with either a severe or mild form of cervicitis and metritis due to infection. Approximately two-thirds showed no clinical evidence of cervicitis and metritis and were negative on bacteriological examination.

4. Most non-infected mares are consistently negative on culture; most infected barren mares are consistently positive on culture.

5. Most mares suffering from uterine infection will fail to conceive when bred.

6. Streptococci are the most common form of infection found as a cause of cervicitis and metritis in mares.

7. *Streptococcus genitalium* lives as a saprophyte in the external genitals of mares. It is found just inside the lips of the vulva of maiden mares and of mares in foal.

8. Of forty mares foaling normally, one-half showed streptococci in uterine secretions when examined from one to nine days after foaling.

9. All cases of metritis from which encapsulated bacilli were isolated were classed as encapsulatus infection even tho other bacteria, including streptococci, were present.

10. On the basis of field and experimental data, encapsulated bacilli causing metritis in mares may be transmitted from mare to mare by the stallion at time of service, the greatest danger being the first few days after serving an infected mare.

11. Stallions do not become permanent carriers of the infection.

12. Metritis of mares due to encapsulated bacilli is differentiated from metritis due to other causes by the character of the exudate and the isolation of the bacillus.

13. In cases of metritis in mares due to infection with streptococci and to encapsulated bacilli, the principal distinguishing feature, in both the clinical and gross pathology, is the character of the exudate.

14. Microscopic sections of uteri from cases of metritis due to streptococci and to encapsulated bacilli show no differential characters of diagnostic value.

15. Mares showing clinical evidence of cervicitis and metritis should, if negative on culture, be re-examined; also mares showing no clinical evidence of infection should, if positive on culture, be reexamined.

16. The streptococcus found in cases of infection of the genital tract of mares, and which has been designated as *Streptococcus genitalium,* is a Gram positive, hemolytic streptococcus. It possesses the fermentative characters generally attributed to the streptococcus pyogenes group. It has been found uniformly virulent for rabbits and non-pathogenic for guinea pigs.

17. The encapsulated bacilli which have been isolated from cases of genital infection in mares have been found to be identical in their morphological, biochemical and serological properties. They have been compared with encapsulated bacilli from human sources, and have been found to belong to the group of organisms designated by Julianelle as Type B. of the Friedlander bacilli. It is noteworthy that most of the strains belonging to this type are of animal origin, while the strains of the other types have been found in infections in humans.

18. Barren mares, free from infection of the genital tract, are more resistant to infection at time of service than are foaling mares.

19. The prevention of streptococcic and other forms of infection in the genital tract of mares is fundamentally a question of sanitation and breeding hygiene.

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